OLYMPUS®

User's Manual

FLUOVIEW FV500

CONFOCAL LASER SCANNING BIOLOGICAL MICROSCOPE

Ver.3.3

Petition

This user's manual is for the software to be run on Olympus FLUOVIEW FV500 Confocal Laser Scanning Biological Microscope. To ensure safety, obtain optimum performance and familiarize yourself fully with this product, we recommend that you study this manual thoroughly before operation.
This user's manual is composed of two volumes including "SYSTEM OVERVIEW", "OPERATION INSTRUCTIONS", "MAINTENANCE" and "TROUBLESHOOTING". Together with this manual, please also read the "User's manual FLUOVIEW FV500" and the instruction manual of the microscope in order to understand overall operation methods. To ensure the safety operation of laser system, we recommend you to study the manual of each laser and the light source equipment besides this manual. Retain this manual in an easily accessible place near a system for future reference.

AX9787

CAUTION

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FLUOVIEW MANUAL CONFIGURATION

The FLUOVIEW system uses two manuals including this “User’s Manual” and the on-screen manual built into the software (“Online Help”).
The User’s Manual is composed of the five following volumes and subject matter:

- SAFETY GUIDE (Anoter manual)
  Describes notes and cautions on using the FLUOVIEW system and on types of warning labels.
- SPECIFICATIONS (Another manual)
  Describes the specifications of the FLUOVIEW system.
- SYSTEM OVERVIEW (This manual)
  Describes the outline of the FLUOVIEW system.
- OPERATION INSTRUCTIONS (This manual)
  Describes the operation procedures of the FLUOVIEW system, for example, methods for image acquisition and various image processing.
- MAINTENANCE (This manual)
  Describes maintenance of the FLUOVIEW system.
- TROUBLESHOOTING (This manual)
  Describes countermeasures in case trouble occurs.

For Online Help, please see “1-3 Online Help” in “OPERATION INSTRUCTIONS” of this manual.

NOTATIONS IN THIS MANUAL

This manual complies with the following notations.

✧ Notation of Caution, Notes and Tips

Notation	Description
	Caution to prevent injuries to the user or damage to the product (including surrounding objects).
	Note for the user.
	Hint or one-point advice for user reference.

✧ Notation of panel, Command Buttons and Dialog Boxes

Notation	Description
[Acquire] panel	The name of a panel, dialog box, list box or check box is enclosed inside square brackets ([ ]).
<OK> button <Open File> button	The name of a button in a panel or dialog box is enclosed inside angled brackets (< >).

✧ Notation of Mouse Operations

Notation	Description
clicking	Action of pressing, then immediately releasing the mouse button.
double-clicking	Action of clicking the mouse button twice in quick succession.
dragging	Action of moving the mouse while holding down the mouse button, then releasing the mouse button at the desired destination.

(Note) In this manual, clicking, double-clicking and dragging involves pressing the left button of the mouse, unless otherwise specified.

✧ Notation of key operations

Notation	Description
Enter	The name of a key is enclosed inside ( ).
Alt + F1	The positive sign (+) expresses the combination of more than one key operation. For example, Alt + F1 refers to pressing the F1 key while holding the Alt key down.
Direction keys	Generic names given to the →, ←, ↑ and ↓ keys.

✧ Notation of system-specific terms

Notation	Description
XY observation	Refers to observation with XY scanning.
(Other observations)	(The same principle also applies to other observations such as XZ, Xt, XYZ, XYt and XYZt.)

Note that some of the panels and dialog boxes shown in this manual are not the precise reproductions of the originals. Some windows are resized to facilitate the reading and some grayed-out characters are printed in readable characters.

SYSTEM OVERVIEW

On This Volume

This volume describes the outline of the FLUOVIEW FV500 system.
Please read this volume so that you can understand the system before use.

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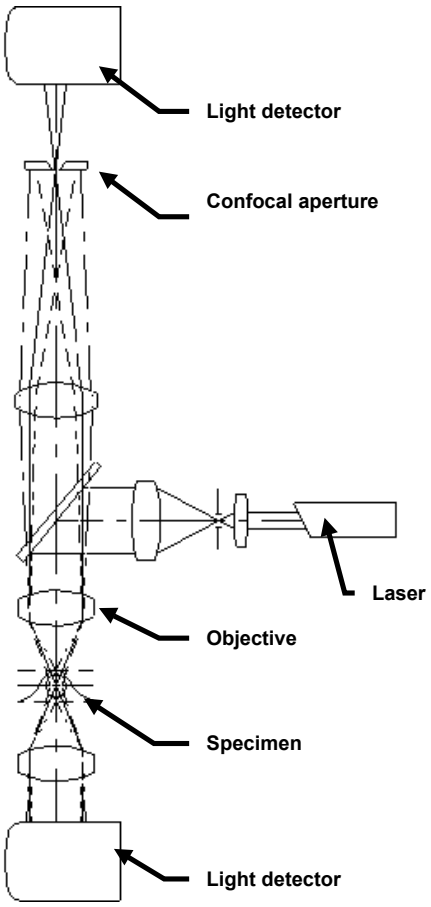
1 SYSTEM OVERVIEW

Olympus FLUOVIEW FV500 is a confocal scanning type laser reflected fluorescence microscope which uses confocal optics for high resolution, high contrast and drastically improved resolution in the light axis direction.
It offers researchers features for sectioning, 3D construction and time-series observation as well as a variety of image processing and analysis functions.

This section describes the principle and characteristics of the system and the functional configuration of software for use with the system.

1-1 Principle

A laser scanning microscope converges a laser beam into a very small spot using an objective and scans the specimen in the X-Y directions.
It then detects the fluorescence and transmitted light from the specimen using light detector and outputs the image of the specimen on an image monitor.
The confocal optics place a confocal aperture at a position which is optically conjugate with the focusing position (i.e. confocal plane) to eliminate light from parts other than those for the focusing position. As a result, the part corresponding to the eliminated light is darkened in the image, making it possible to slice a thick tissue specimen optically.
On the other hand, with ordinary optical microscopes, light from a part other than the focusing position overlaps with the image-forming light on the focusing position, so the overall image tends to be vague.
Transmitted light images can be obtained by use of a transmitted light detector, but the images will not be confocal images, because there is no confocal aperture in front of the detector.



The transmitted light image can be used by combination with a confocal fluorescence image to observe fluorescent localization.

1-2 Features of FLUOVIEW FV500

1. The detector has 12-bit resolution and is capable of detecting very small changes in fluorescence inside cells.

2. The system has high resolution of 2048 x 2048 pixels. The output video signal is non-interlaced for clear images without flickering.

3. The transmitted light is detected by a photomultiplier for bright, sharp transmitted images.

4. The auto gain adjustment feature eliminates the need for complicated gain adjustment operation.

5. A total of 5 image channels, including up to 4 fluorescence images and a transmitted image, can be acquired simultaneously.

6. The scan unit can be used in common with an upright or inverted microscope.

7. The confocal aperture is continuously variable and the optimum confocal aperture for each objective is set automatically.

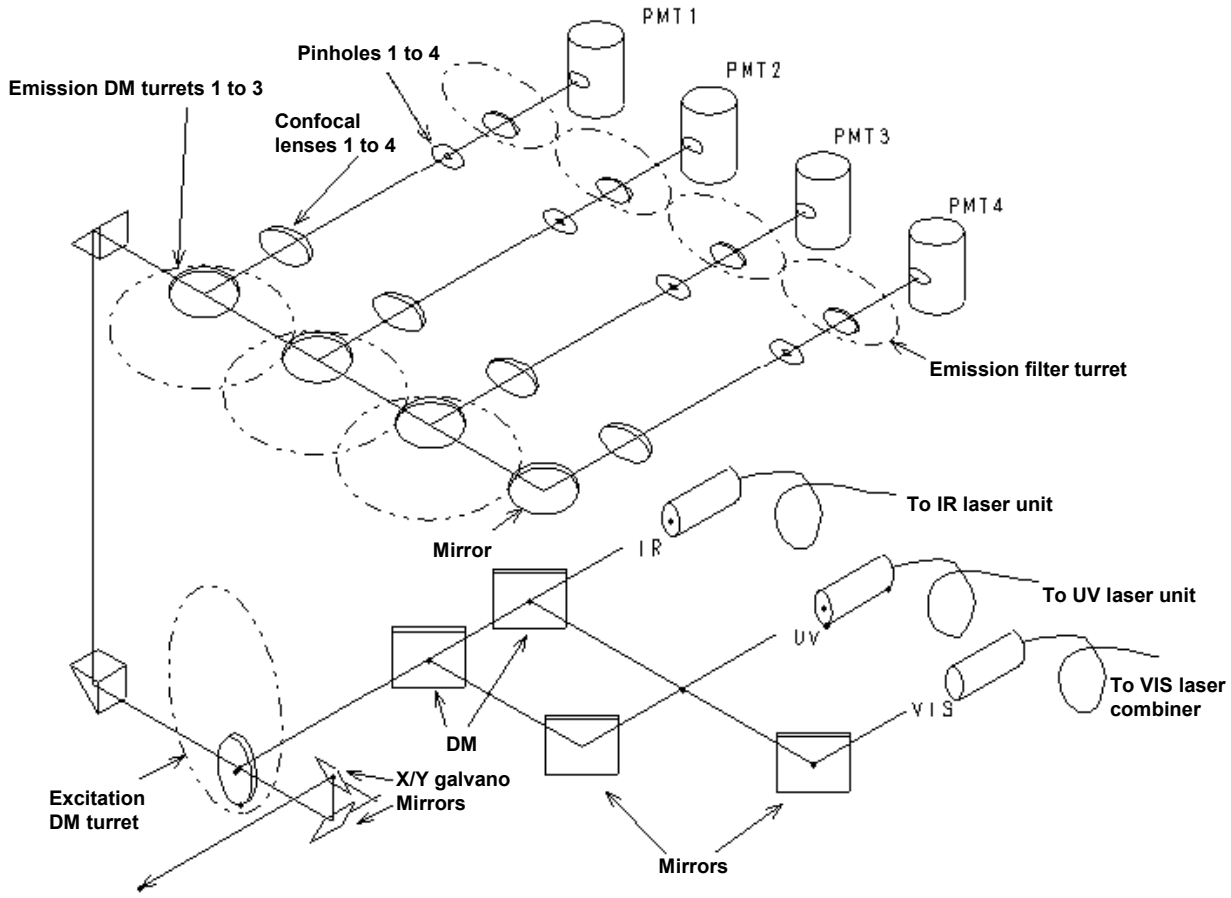
8. High-speed scanning acquires 4 images per second (512 x 512 pixels).

9. Sequential scan for acquiring images without crossover.

10. In addition to raster scanning, scanning modes such as vector scanning and oblique scanning are provided to meet a wide range of applications.
11. The dichroic mirror for excitation, dichroic mirror for spectroscopy, confocal aperture and emission filters are set automatically.

12. When using the system with AOTF (FV5-COMBA), the following functions are available.
    - Line Kalman scanning
    - Line sequential scanning
    - Image acquisition in the REX/Bleach mode(FRAP experiment)

1-3 Light Path Diagram



1-4 System Configuration

1-4-1 System Component Units and Their Functions

Laser combiner
Unit for merging laser beams from the lasers such as an Ar laser, Kr laser and HeNe laser into a single beam to be transmitted through the optical fiber cable.

Scan unit
Heart of a laser microscope, composed of the light scanner and light detector.

Transmitted light detector unit
Unit for acquiring transmitted images. Connected with the microscope through an optical fiber cable.

Microscope
AX70 upright microscope set up for fluorescence observation.

Z-motor
Motor for driving the focusing block of the microscope

Multi-control box
Controller of the AX70 microscope.

Transmitted light unit
Unit incorporating a 100 W halogen lamp bulb to provide illumination for transmitted light observation. Connected with the microscope through an optical fiber cable.

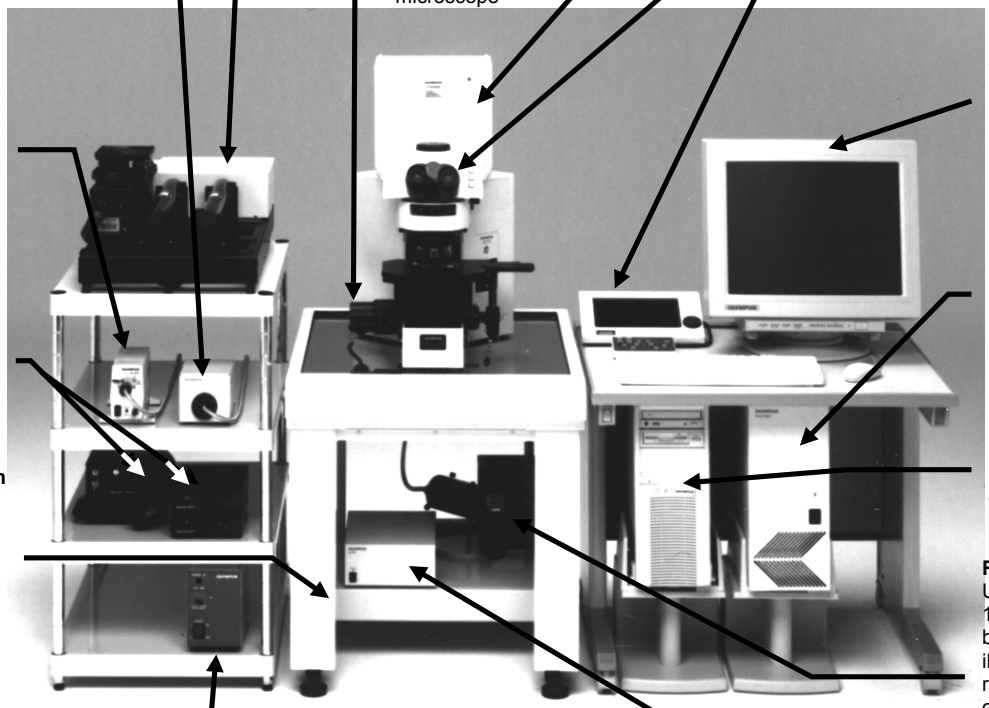


Image monitor
Video monitor for displaying laser scanning images, control panel, etc.

Laser power supply units

Control unit
Unit for driving the scanning unit, laser combiner, X-motor and transmitted light detector.

Computer
Used to control the LSM, process image files, etc.

Anti-vibration platform
Exclusively designed pneumatic platform.

Reflected light unit
Unit incorporating a 100 W mercury burner to provide illumination for reflected light observation. Connected with the microscope through an optical fiber cable.

Reflected Fluorescence Light Power Supply Unit
Power supply unit for the mercury burner for reflected light observation.

Photo 1-1 Combination with AX70

Power box
Power supply unit for the AX70 microscope.

Microscope
Upright microscope exclusively designed for FLUOVIEW FV500.

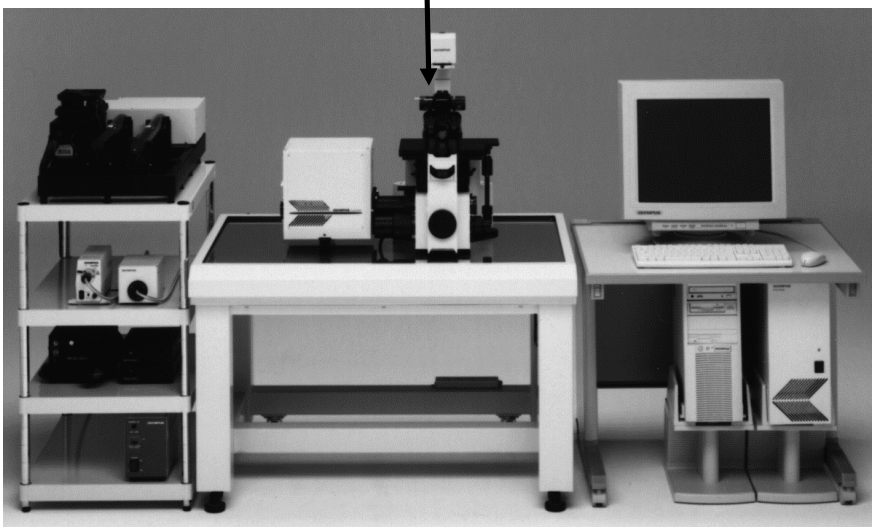
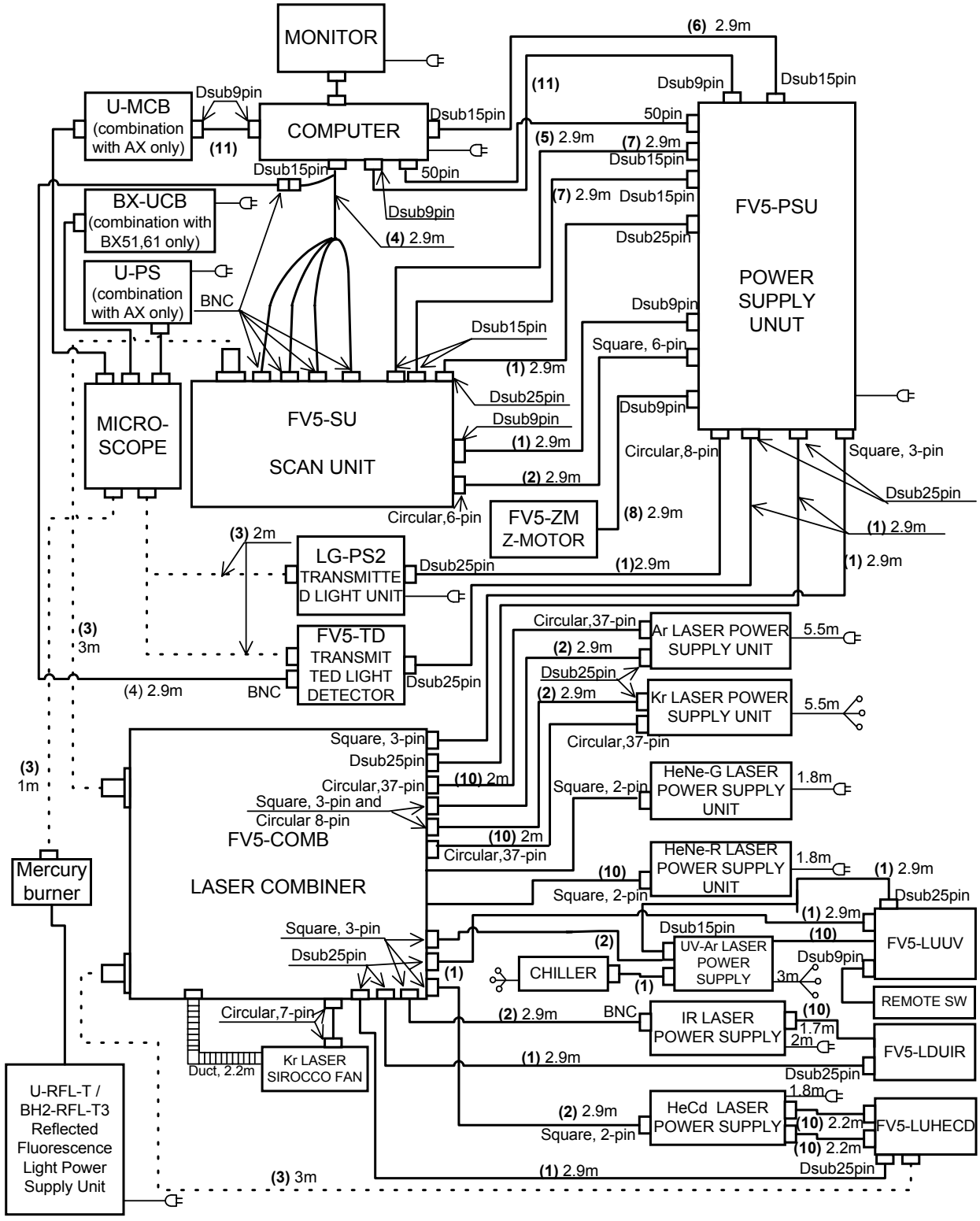
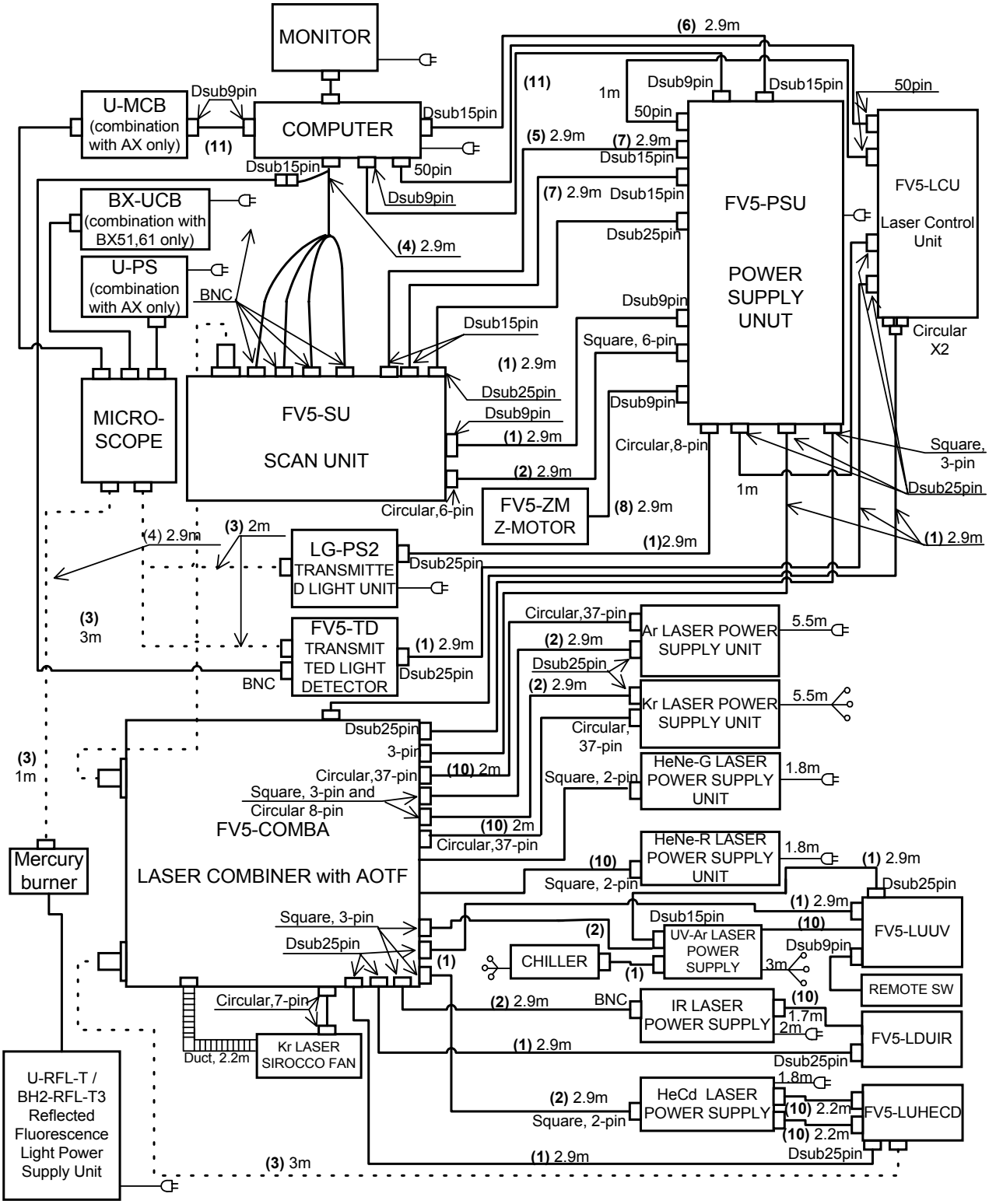


Photo 1-2 Combination with IX

1-4-2 Block Diagram



The block diagram in AOTF (FV-COMBA) use.



Either the Kr laser or HeNe-G laser can be used.

(1) Control cable

(2) Interlock cable

(3) Optical fiber cable

(4) Analog signal cable

(5) I/O cable

(6) Galvano control cable

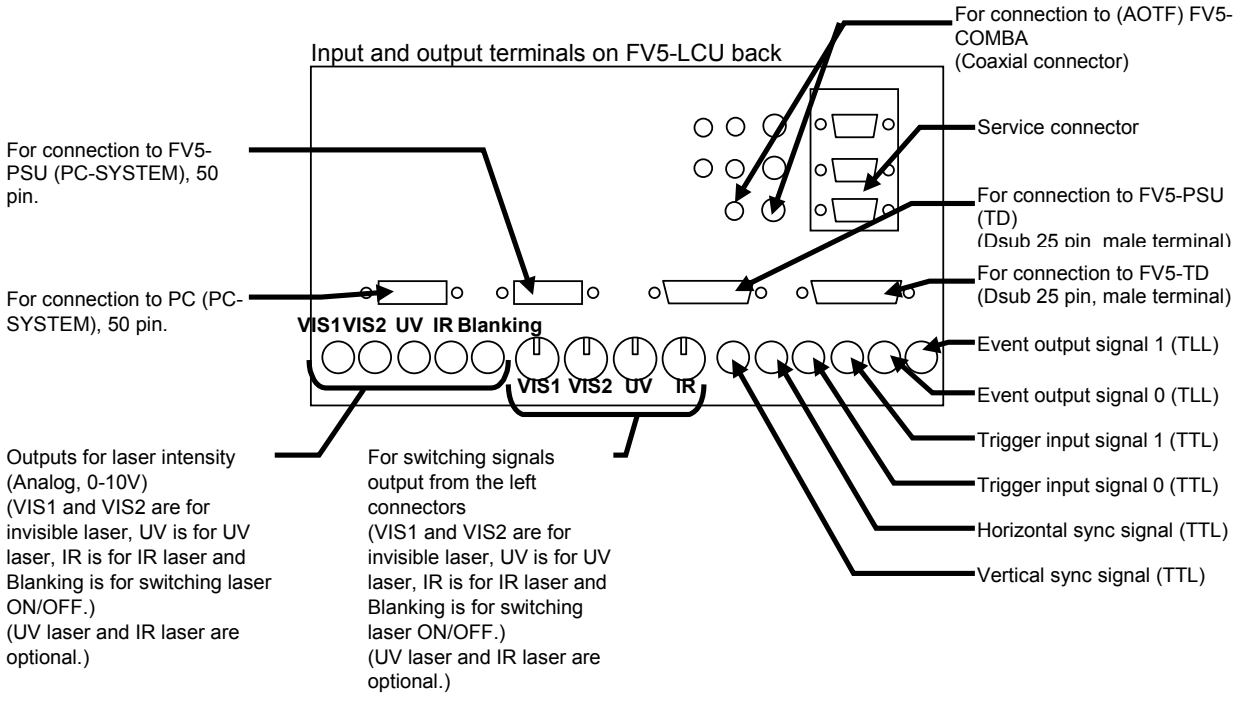
(7) Galvano X/Y cable

(8) Power/control cable

(9) Power cable

(10) Laser power cable

(11) RS-232C cable



1-5 Software Functional Configuration

This software uses panel-type windows.

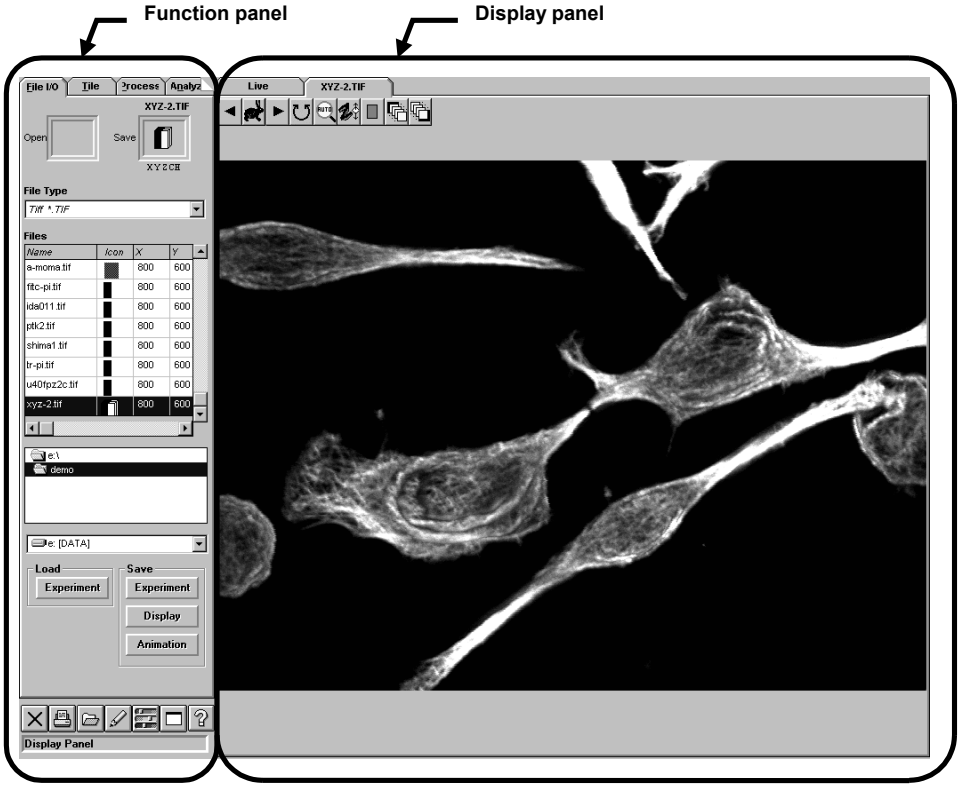
Usually, it is required to “select a menu then select the command to be executed” in order to execute a function provided by software. With the panel system, a software function can be executed easily by “selecting the panel page tab of the function to be executed”, just like when using a system notebook or file folder.

1-5-1 Function Panel and Display Panel

The FLUOVIEW software is organized by two kinds of panels, the function panels and display panel.

The function panels include the [Acquire], [File I/O], [Tile], [Process], [Analyze] and [Visualize] panels.

The display panel shows either the [Live] panel or the panel image loaded from a file ([(filename)] panel).



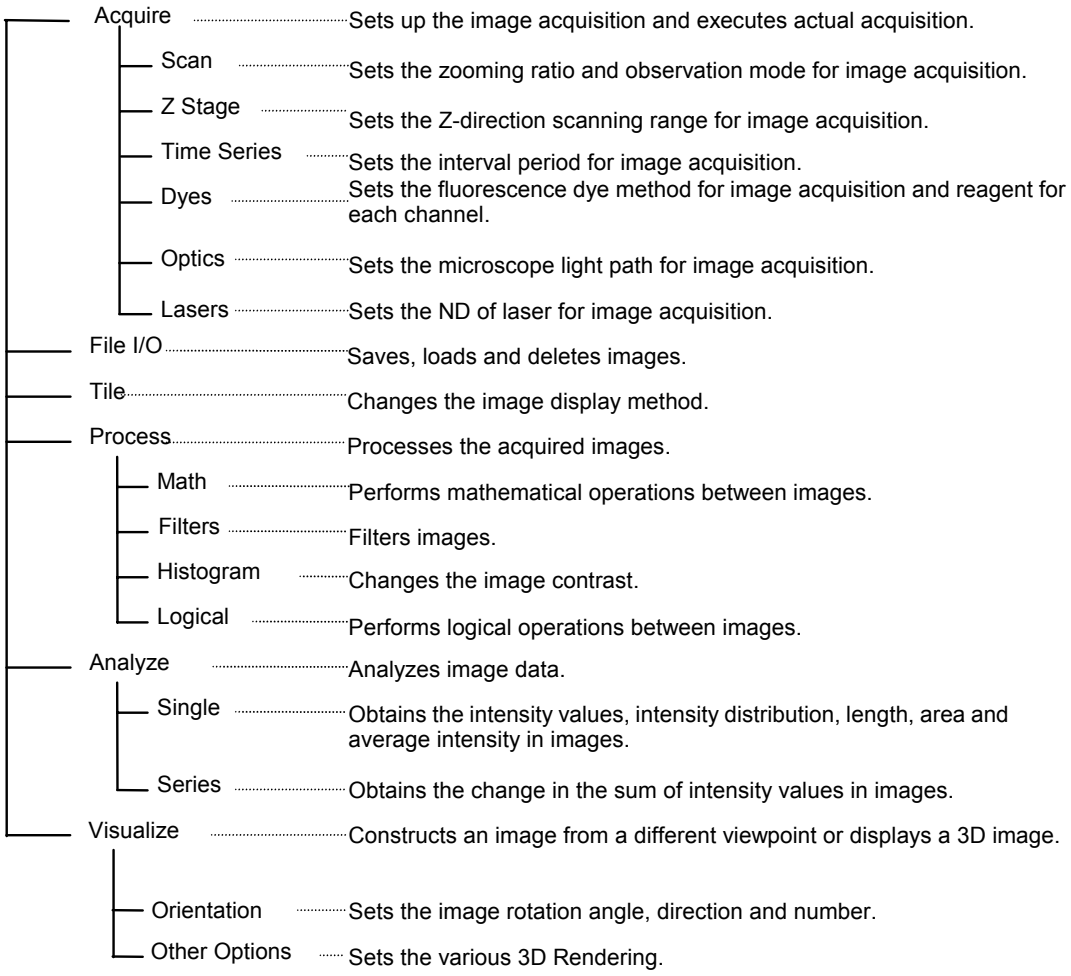
NOTE

In this manual, the function panels are referred to simply using their page tabs.

Namely, the [File I/O] panel of function panel is referred to simply as the [File I/O] panel.

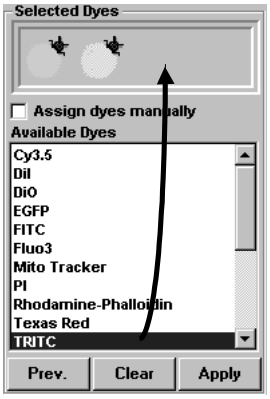
1-5-2 Panel Structure of the Software

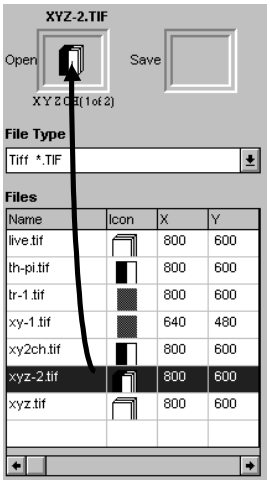
This software cannot show the indexes of all function panels at a glance, but uses scrolling to display the desired page tab. Please use the following list of the panels for reference in scrolling.

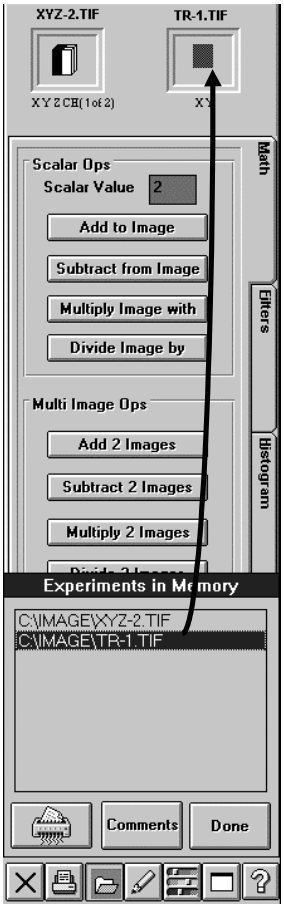


1-5-3 Icons Executed by Dragging & Dropping

This software selects image files and observation methods (dye name) by means of dragging & dropping. This allows simple selection based on an intuitive operation of "selecting an icon (image file or observation method), dragging it to the desired position and dropping it there".







1-5-4 Identification of Images Depending on the Observation Methods

Image Icon	Significance
	XZ observation
	XZ observation, 2-channel mode
	Xt observation
	Xt observation, 2-channel mode
	XZT observation
	XZT observation, 2-channel mode
	XY observation
	XY observation, 2-channel mode
	XYt observation
	XYt observation, 2-channel mode
	XYZ observation
	XYZ observation, 2-channel mode
	XYZt observation
	XYZt observation, 2-channel mode
	Point Scan
	Animation image
	Stereo 3D image: Image to be viewed with color eyeglasses.
	3 or more channels

On many occasions, FLUOVIEW displays image icons to allow identification of the observation method used when each image is acquired. (See table on the left.)
When the [File I/O], [Tile], [Process], [Analyze] or [Visualize] panel is selected, the icon of the image selected in the [Display] panel is displayed in a frame at the top of the function panel. The image icons are also displayed in the [Icon] field in the [Files] list box in the [File I/O] panel or during dragging of an image file. Use these icons to identify the observation methods used in image acquisition.



In all observation modes, the icons for 3 or more channels are identical.

OPERATION INSTRUCTIONS

On This Volume

This volume describes the operating procedures of the FLUOVIEW FV500 system.
“Getting Started FLUOVIEW” contains information on the basic operation flow until acquisition of XY images.
“APPLIED OPERATIONS” provides detailed operating procedures of the system.
Please read this volume so that you can understand the system before use.

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1 Getting Started FLUOVIEW

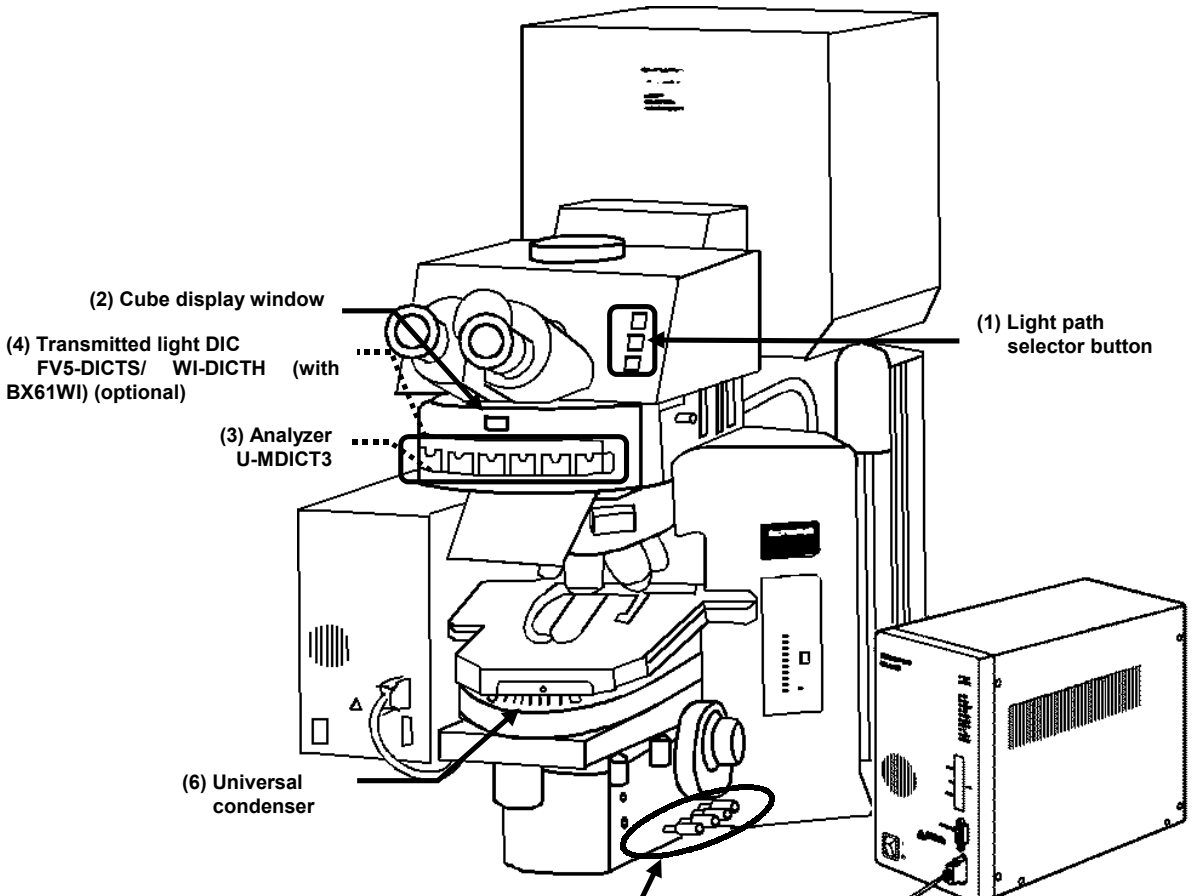
1-1 Basic Operations

1-1-1 Microscope

The following figure shows the major controls of a microscope. The actual configuration of the modules including the specimen stage, revolving nosepiece and lighting equipment may differ from those shown below.

For detailed microscope operation procedures, refer to the instruction manuals of your microscope.

Combination with BX51 or BX61



(1) Light path selector button

Select the light path between the visual observation and laser microscopy.

- Select the < > button for visual observation.
- Select the <1> button for TV or photomicrography.
- Select the <2> button for laser microscopy.

(2) Cube turret

Select the fluorescence observation tube by rotating the turret.

- Engage the desired cube in the light path for visual fluorescence observation.
- For laser microscopy or visual transmitted light observation, rotate the turret to page tab . (This sets the cube turret so that no cube is engaged.)

(3) Analyzer U-MDICT3

Polarizing plate for use in differential interference observation and polarized light observation.

- Rotate the turret to engage the U-MDICT3 in the light path for visual transmitted light differential interference observation or transmitted polarized light observation.
- For laser microscope, always disengage the U-MDICT3. Engaging the U-MDICT3 in the light path degrades the image quality.

(4) Transmitted light DIC FV5-DICTS/WI-DICTHRA (when using BX61WI) (optional)

This is the prism for use in differential interference observation.

- Engage the transmitted light DIC in the light path for laser differential interference observation or visual transmitted light differential interference observation.

  Leaving the transmitted light DIC engaged during laser fluorescence observation will degrade the resolution somewhat. We recommend disengaging the transmitted light DIC from the light path when simple laser fluorescence observation is required.

NOTE

With transmitted light differential interference observation using an immersion objective, set the microscope's field diaphragm so that it circumscribes the field of view. Otherwise the contrast may degrade. (This applies to both visual observation and laser differential interference observation.)

(5) Filters

These filters are used to adjust the transmitted light.

- Be sure to disengage any filter from the light path for transmitted observation using lasers. Leaving a filter engaged in the light path will degrade the image quality.

  When you perform transmitted observation using laser with BX61WI, use the filter knob to disengage the LBD from the light path and engage the FR (Frost) into the light path. Disengaging the FR (Frost) from the light path may generate interference fringes on an image.

(6) Universal condenser

Condenser for transmitted lighting. In addition, the rotary turret for the transmitted light DIC prism and the polarizing plate for differential interference observation (polarizer) are also provided.

- To perform differential interference observation, engage the transmitted light DIC prism matching the objective in use in the light path (For both visual observation and laser differential interference observation).
- To perform visual differential interference observation or laser differential interference observation, engage the polarizing plate in the light path.

(7) Hand switch U-HSTR2 (U-FH is optionally available with BX61)

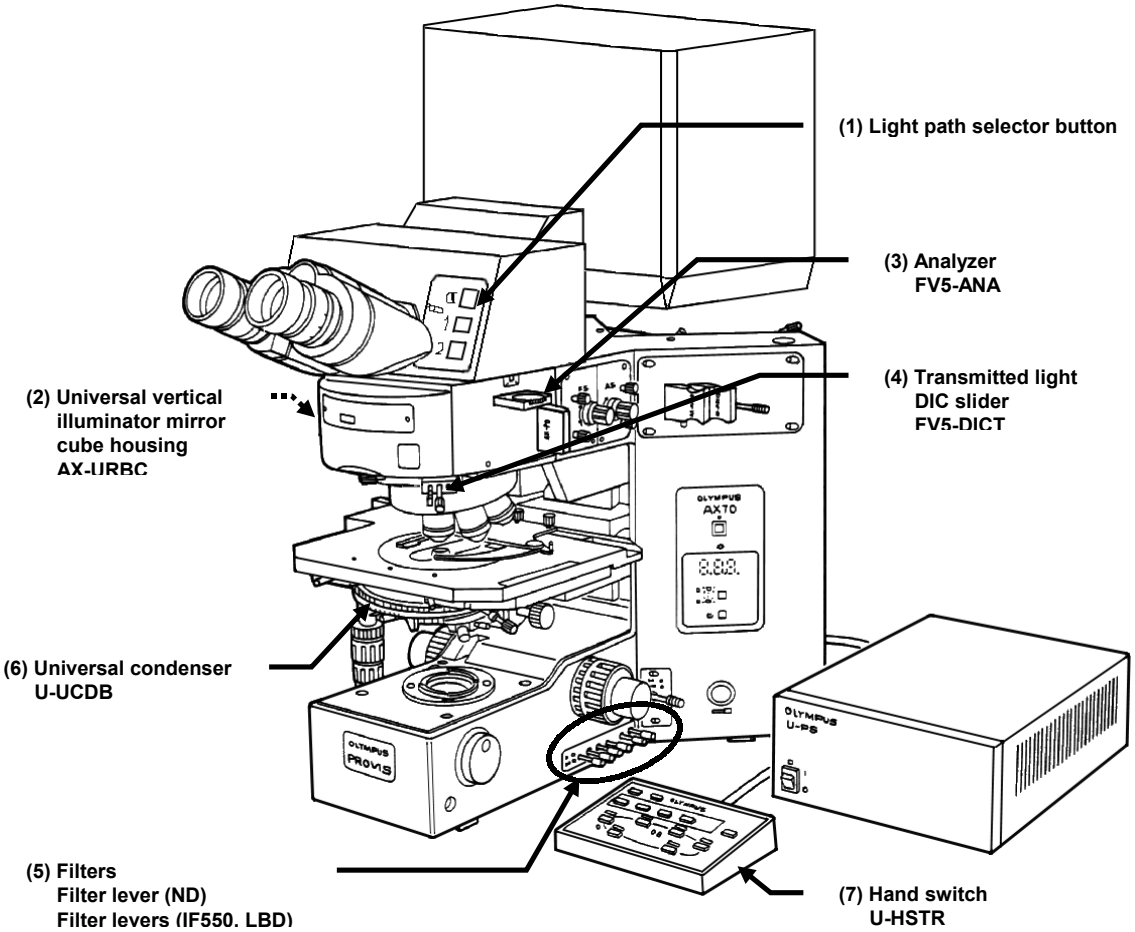
The hand switch corresponding to the BX electric system.

NOTE

Connect the filter wheel of BX to connector of the following in the back of UCB.

- **FW0: FW1**
- **FWR: FW2**
- **FWT: FW3**

Combination with AX70 or AX70WI



(1) Light path selector button

Select the light path between the visual observation and laser microscopy.

- Select the < ◐ > button for visual observation.
- Select the <1> button for TV or photomicrography.
- Select the <2> button for laser microscopy.

(2) Universal vertical illuminator mirror cube housing

The turret for the cube for reflected light observation can be switched with the hand switch for transmitted/reflected light (7).

- Engage the desired cube in the light path for visual fluorescence observation.
- For laser microscopy or visual transmitted light observation, press the BF cube switch button of the hand switch (7). (This set the cube turret so that no cube is engaged.)

(3) Analyzer FV5-ANA

Polarizing plate for use in differential interference observation and polarized light observation.

- Set to the pushed-in position to engage the FV5-ANA in the light path for visual transmitted light differential interference observation or transmitted polarized light observation.
- For laser microscope, set to the pulled-out position to disengage the FV5-ANA from the light path. Engaging the FV5-ANA in the light path degrades image quality.

(4) Transmitted light DIC slider FV5-DICT

This is a prism for use in differential interference observation.

- Engage the FV5-DICT in the light path for laser differential interference observation or visual transmitted light differential interference observation.
  Leaving the FV5-DICT engaged during laser fluorescence observation will degrade resolution somewhat. We recommend disengaging the FV5-DICT from the light path when simple laser fluorescence observation is required.



With transmitted light differential interference observation using an immersion objective, set the microscope's field diaphragm so that it circumscribes the field of view. Otherwise the contrast may degrade. (This applies to both visual observation and laser differential interference observation.)

(5) Filters

These filters are used to adjust transmitted light.

- Be sure to disengage any filter from the light path for transmitted observation using lasers. Leaving a filter engaged in the light path will degrade the image quality.

(6) Universal condenser U-UCDB

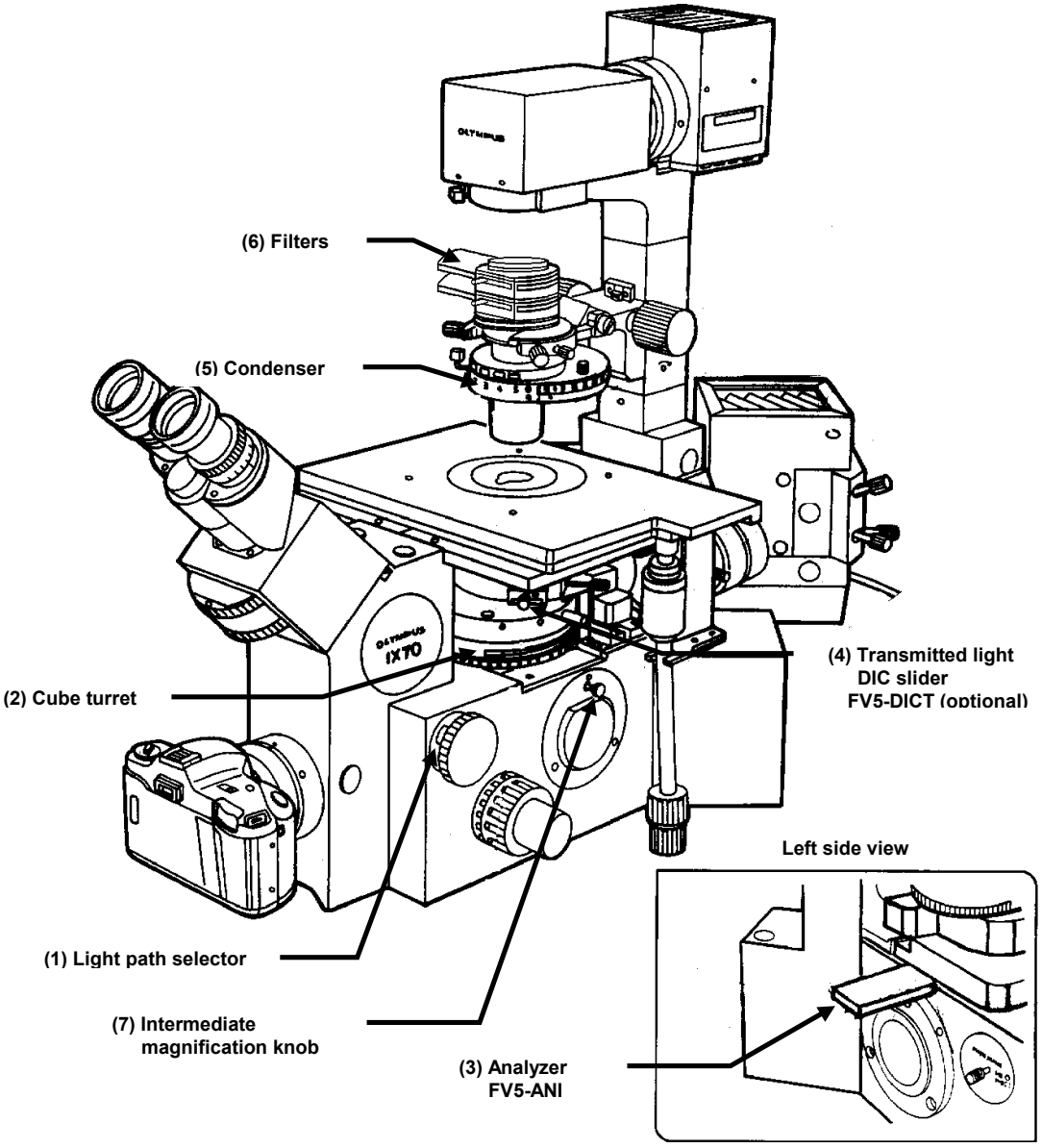
Condenser for transmitted lighting. In addition, the rotary turret for the transmitted light DIC prism and the polarizing plate for differential interference observation (polarizer) are also provided.

- To perform differential interference observation, engage the transmitted light DIC prism matching the objective in use in the light path. (For both visual observation and laser differential interference observation)
- To perform visual differential interference observation or laser differential interference observation, engage the polarizing plate in the light path.

(7) Hand switch U-HSTR

Switches the objectives and cubes.

Combination with IX



(1) Light path selector

Select the light path between laser microscopic observation, direct observation and photography observation.

- Refer to the following table and set the dial switch to the position corresponding to the required light path.

<<Light intensity of each light path>>

Light Path \ Dial Position			
Side port (LSM)	-	-	100%
SLR port	-	100%	-
Binocular section	100%	-	-

(2) Cube turret

Select the fluorescence observation tube by rotating the turret.

- Engage the desired cube in the light path for visual fluorescence observation.
- For laser microscopy or for visual transmitted light observation, rotate the turret to page tab  . (This set the cube turret so that no cube is engaged.)

(3) Analyzer FV5-ANI

Polarizing plate for use in differential interference observation and polarized light observation.

- Set to the pushed-in position to engage the FV5-ANI in the light path for visual transmitted light differential interference observation or transmitted polarized light observation.
- Set to the pulled-out position to disengage the FV5-ANI for laser microscopy. Engaging the FV5-ANI in the light path degrades image quality.

(4) Transmitted light DIC slider FV5-DICT (optional)

This is a prism for use in differential interference observation.

- Engage the FV5-DICT in the light path for laser differential interference observation or visual transmitted light differential interference observation.
  Leaving the FV5-DICT engaged during laser fluorescence observation will degrade image quality somewhat. We recommend disengaging the FV5-DICT from the light path when simple laser fluorescence observation is required.

NOTE **With transmitted light differential interference observation using an immersion objective, set the microscope's field diaphragm so that it inscribes the field of view. Otherwise the contrast may degrade. (This applies to both visual observation and laser differential interference observation.)**

(5) Condenser, polarizing plate

Condenser for transmitted lighting.

In addition, the rotary turret for the transmitted light DIC slider and the polarizing plate for differential interference observation (analyzer) are also provided.

- To perform differential interference observation, engage the transmitted light DIC slider (optional) matching the objective in use in the light path (For both visual observation and laser differential interference observation).
- To perform visual differential interference observation or laser differential interference observation, engage the polarizing plate in the light path.

(6) Filters

These filters are used to adjust transmitted light.

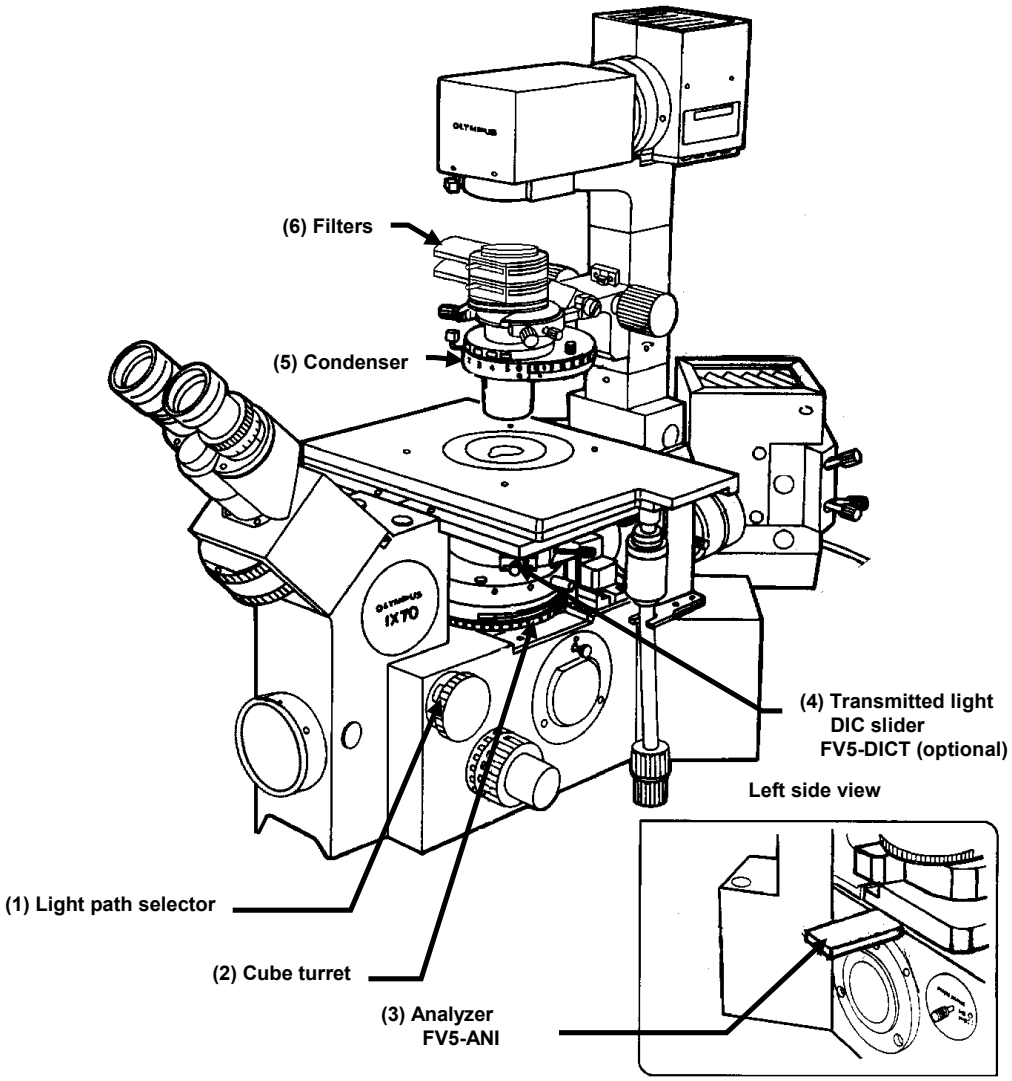
- For transmitted observation using laser, disengage the LBD filter from the light path and engage the FR (frost) filter in the light path by operating the filter levers. If the FR filter is disengaged from the light path, the image may suffer from stripe interference.

(7) Intermediate magnification knob

Select the intermediate magnification from 1X or 1.5X.

- Always set to 1X for using the microscope as a LSM. It cannot be used as a LSM if this knob is set to 1.5X.

Combination of IXBP (Bottom port)



(1) Light path selector knob

Switch the light path between laser microscope observation, visual observation and side port.

- Set the knob to the position for the desired light path by referring to the following table.

«Light ratios of light paths»

Knob Position Indication / Light Path	LSM	SP
Side port	-	100%
Binocular tube or LSM	100%	-

(2) Cube turret

Switch the cubes for fluorescence observation by rotating this turret.

- For visual fluorescence observation, engage the specified cube in the light path.
- For laser microscopy or for visual transmitted light observation, rotate the turret so that the index indicates ◯. (This sets the cube turret to an idle position without cube.)

(3) Analyzer FV5-ANI

Polarizer for use in DIC or polarized light observation.

- For visual transmitted DIC observation or transmitted polarized light observation, push in the lever to engage the analyzer in the light path.
- For laser microscopy, be sure to disengage the analyzer from the light path by pulling the lever out. High quality image cannot be obtained if the analyzer is left engaged in the light path.

(4) Transmitted light DIC slider FV5-DICT (optional)

Prism for use in DIC observation.

- For laser DIC observation or visual transmitted light DIC observation, engage If fluorescence observation using laser is performed with the FV5-DICT engaged in the light path, the resolution may deteriorate somewhat. It is recommended to disengage the FV5-DICT from the light path when performing only the fluorescence observation using laser.

NOTE

With transmitted DIC observation using a water-immersion objective, reduce the field iris diaphragm of the microscope until it circumscribes the field of view. Otherwise, the contrast may deteriorate. (This applies to both visual and laser DIC observations.)

(5) Condenser and polarizer

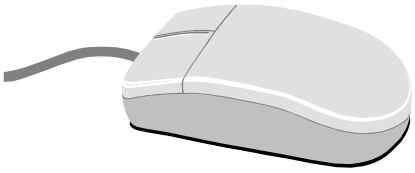
Condenser for transmitted light. The rotary turret for the transmitted light DIC slider and the polarizer for DIC observation are also provided here.

- For DIC observation, engage the transmitted light DIC slider (optional) matching the objective in use in the light path. (This applies to both visual and laser DIC observations.)
- For visual DIC observation or laser DIC observation, engage the polarizer in the light path.

(6) Filter

Used in adjustment of the transmitted light illumination.

1-1-2 General Mouse Operation Procedures



Use the mouse to select a command, character string or button. Use the left button of the mouse unless otherwise specified.

To select or execute something: Clicking

To click the mouse, place the mouse pointer on the desired function and press the mouse button once.
(Pressing the right button of the mouse is referred to as right-clicking.)

To select something and execute its function: Double clicking

To double-click, place the mouse pointer on the desired function and press the mouse button successively twice.

To move something: Dragging

To drag, place the mouse pointer on the desired function, and while pressing and holding the mouse button, move the mouse to the desired destination. At the desired destination, release the mouse button.
(Dragging by pressing the right button of the mouse is referred to as right-dragging.)

One Point!

When the mouse is moved, the picture of arrow on the screen moves accordingly. The picture which moves on the screen as the mouse is moved is referred to as the mouse pointer.

1-1-3 Names of Major Panel and Window Controls and Their Functions

The window as shown below is displayed when FLUOVIEW starts up. FLUOVIEW uses panel-type windows.

This section describes the names of the major controls displayed in panels and windows by taking the [Acquire] panel and [Microscope Configuration] window as examples.

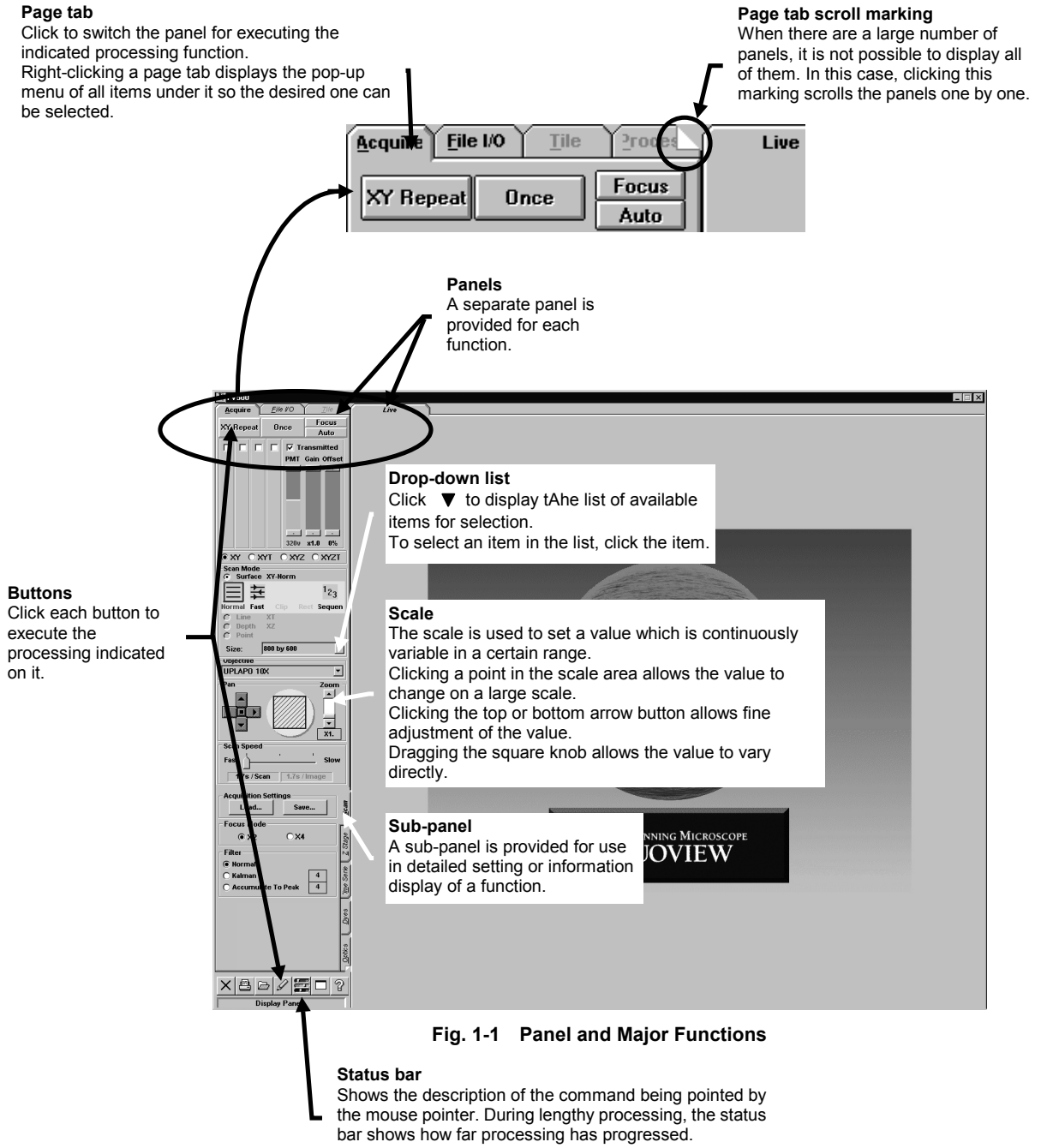
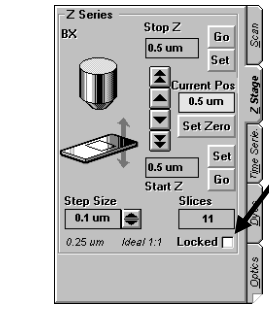


Fig. 1-1 Panel and Major Functions



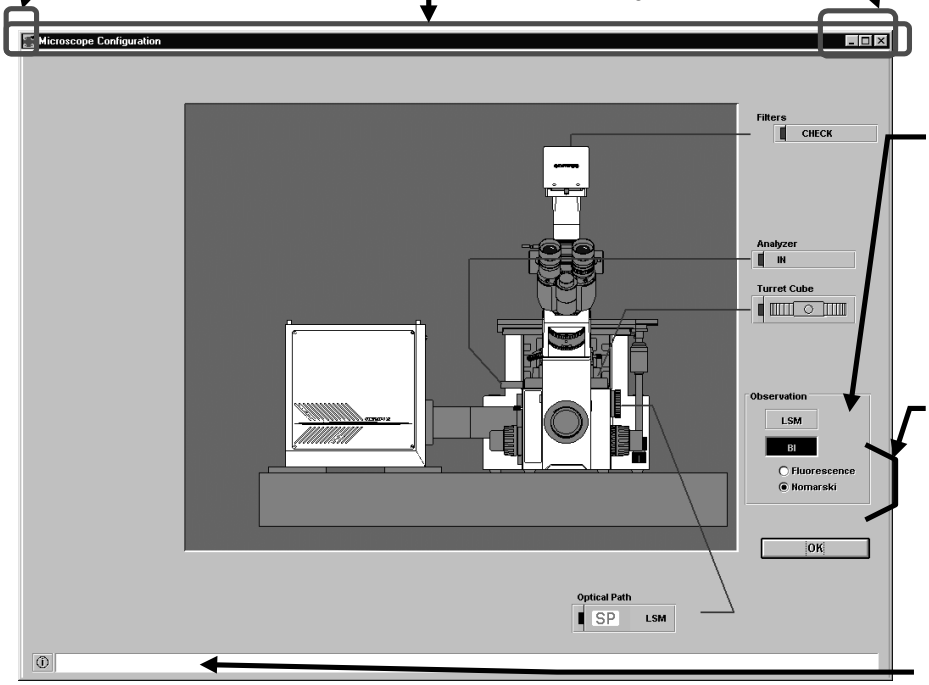
Check box
Clicking this box enables or disables the indicated item. The item is enabled when the check box is checked (x).

Title bar
Shows the title of the window. The title bar of a window that is active is displayed in a different color from that of other windows.

Control menu box
Clicking this box displays a control menu, which contains the commands for use in controlling the window.

Minimize button <>
Click to turn the window into an icon.

Original size button <>
Click to return the maxim-size window to its original size.

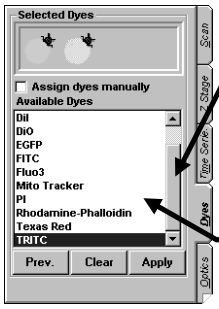


Group box
The group box groups functions with specific meanings and encloses them in a frame.

Option buttons
This is a group of multiple items among which only one can be selected.
Clicking one of the round buttons selects the corresponding item.
The option button of the selected item is displayed with a black dot in the center of it.

Information
Shows information on the operations and meanings of functions.

Fig. 1-2 Window and Major Functions

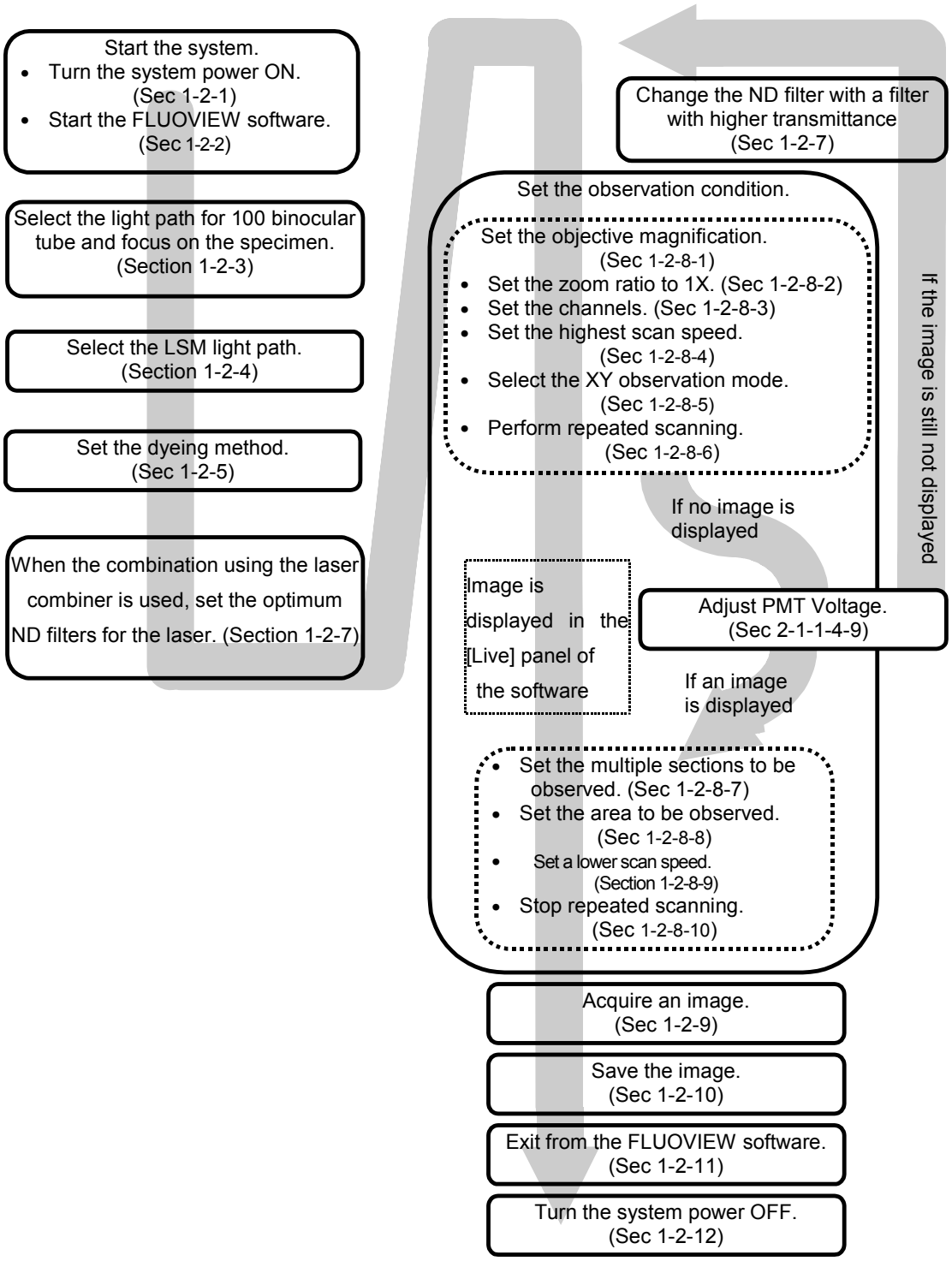


Scroll bar
The scroll bar is displayed when there are too many data items to be displayed in a field at once, and is used to display the data items outside the field. Clicking a point in the scroll area allows data items to be scrolled in large steps.
Clicking the top or bottom arrow button allows fine scrolling of the data items.
Dragging the square knob allows direct scrolling.

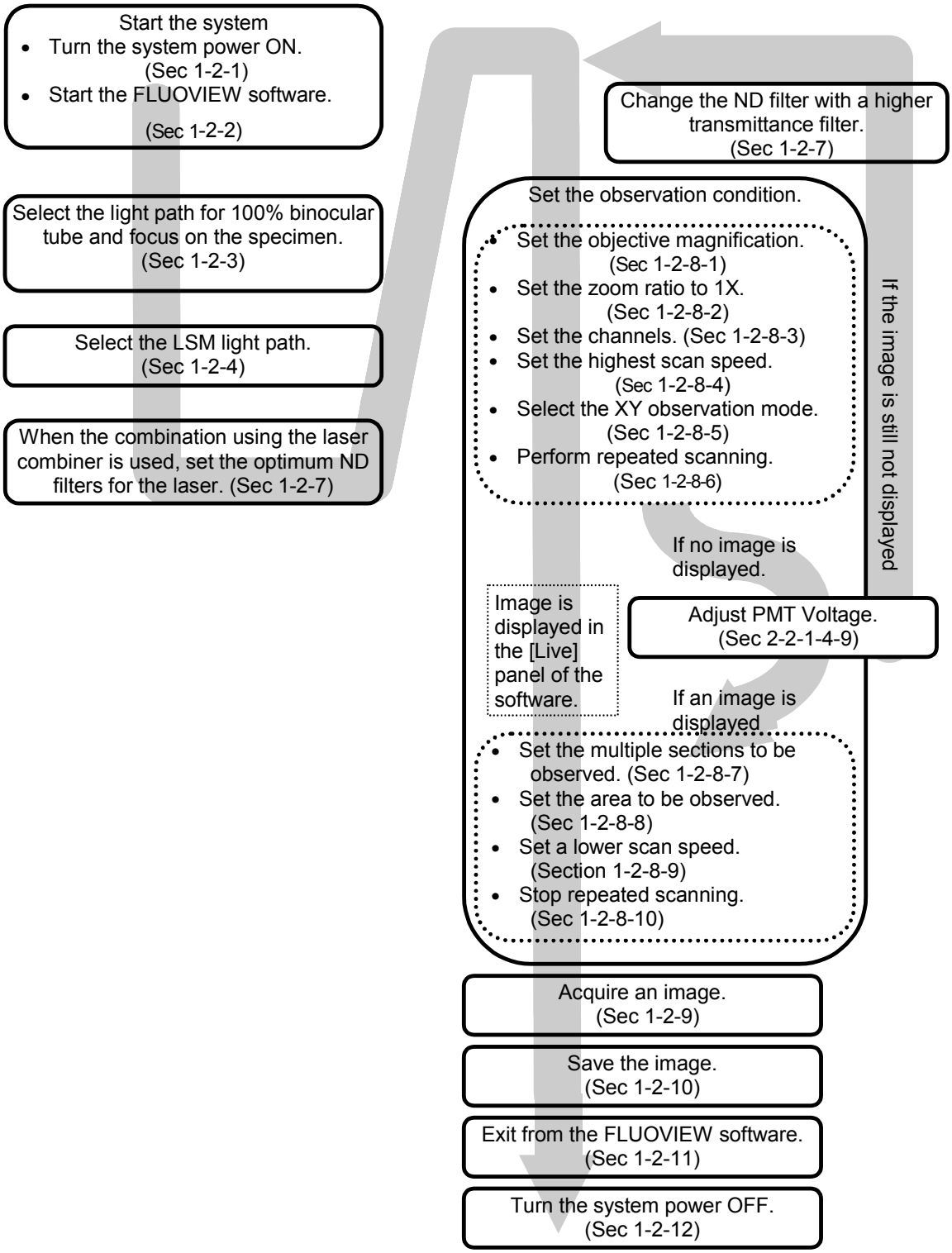
List box
Shows the list of available items for selection. All items in the list can be displayed by scrolling. To select an item in the list, double-click or drag the item.

1-2 Outline of LSM Observation Procedures

- **Fluorescence observation procedure**



- **Transmitted light observation procedure**



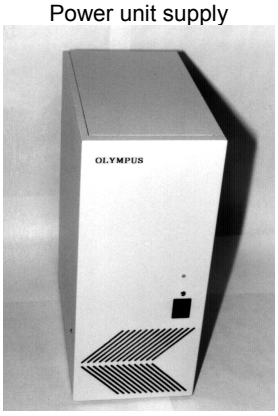
1-2-1 Turning Power On

Set the power switch of each unit to ON.

As the computer, monitor, power unit and hard-copy unit (optional) are connected to the switched power outlet unit, their power is turned ON or OFF simultaneously according to the power ON/OFF of the computer.

When it is required only to read data, it is not required to turn the laser power supply and reflected light power supply units ON.

Power unit supply



1. Set the power switch of the computer (PC) ON

   The power to the following units is turned ON in interlocking with the power of the computer.

   • Power supply unit

   • Monitor

   (Refer to the instruction manual of your monitor.)

   Since these units are permanently connected to the computer, leave their power switches set to the ON position.

NOTE

It takes about 2 minutes after power supply unit turns ON until it is initialized.

Before proceeding to any other operation, wait for about 2 minutes after turning power ON.

Ar laser supply



2. Turning the laser power ON

   2.1.Ar laser:

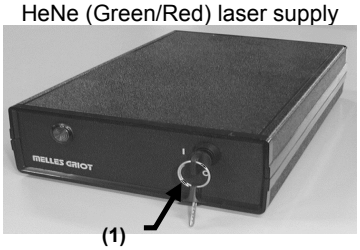
   (1) Set the power switch to ON.

   (2) Turn the key to the ON position. (The laser fan will start.)

   (3) Turn the key further in the clockwise direction. (The key will return to the ON position.)

   The laser starts oscillation in some tens of seconds after the key is set to ON.

HeNe (Green/Red) laser supply



2.2. Kr laser:

(1) Set the power switch to ON.

(2) Turn the key to the ON position. (The laser fan will start.)

(3) Turn the key further in the clockwise direction. (The key will return to the ON position.) After the key is set to ON, it takes tens of seconds before the laser oscillation starts.

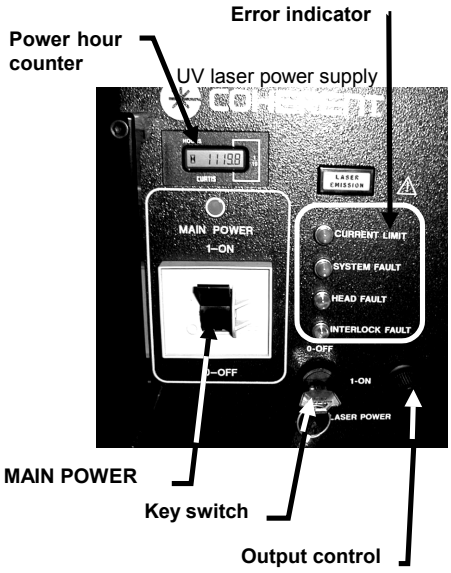
2.3. HeNe (Green/Red) laser:

(1) Turn the key to the "I" (ON) position.

After the key is set to ON, it takes tens of seconds before the laser oscillation starts.

NOTE

To stabilize the laser beam output, we recommend leaving the system for warm-up after turning the laser power ON. The warm-up period should exceed 10 minutes when the laser system in use uses an Ar laser or exceed 30 minutes when it uses a Kr laser or Green HeNe laser.



2.4 UV-Ar laser

When using the system for the first time, check that the remote cable is connected, the [POWER] switch on the front panel of the chiller is set to "O-OFF", its [COOL DOWN CYCLE] switch is set to "I-ON" and the remote operation mode is set.

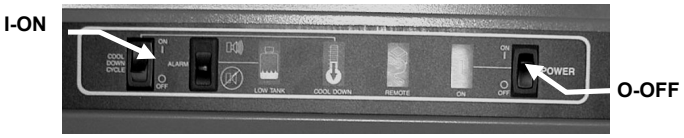


Fig. 1-3 Chiller Front Panel

From the second time and on, use the following method.

(1) Set [MAIN POWER] of the laser power supply to ON and turn the key switch to the "I-ON" position.

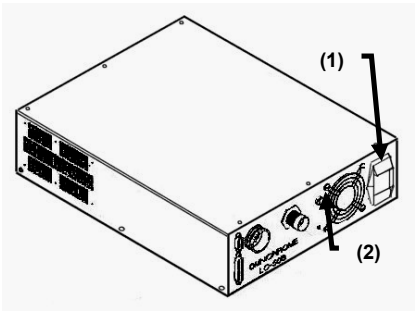
(It takes about 30 seconds before the laser starts oscillation together with a trigger sound. All error indicators turn off at this time.)

(2) Make sure that the output control is set to the maximum position.

(3) Warm up for about 30 minutes until the oscillation stabilizes.

(4) Open the shutter lever of the laser tube.

2.5. For the method of turning an IR (750 nm) laser on, refer to the instruction manual of the IR laser (by MELLESGRIOT).



Laser controller

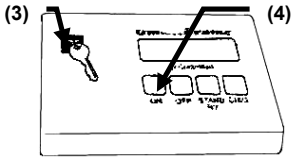
2.6. HeCd laser.

(1) Turn the rocker switch(1) of laser controller to “I” (ON).

(2) Turn the key switch(2) of laser controller to the ON position.

(3) Turn the key switch(3) of remote interface module to the ON position.

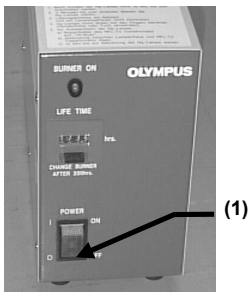
(4) Turn the <ON> button of the remote interface module ON. After the key is set to ON, it takes tens of seconds before the laser oscillation starts.



Remote interface module

NOTE

To stabilize the laser beam output, we recommend leaving the system for warm-up after turning the laser power ON. The warm-up period should exceed 10 minutes when the laser system in use uses an Ar laser or exceed 30 minutes when it uses a Kr laser, Green HeNe laser or HeCd laser.



3. Turning the reflected light power supply unit

(1) Set the power switch to ON.

Once the reflected light power supply unit is turned OFF, do not turn it ON again for at least 10 minutes. Otherwise the service life of the mercury burner will be shortened.

- Turning the computer (PC) ON

  When the power outlet unit is used, turning the computer power ON also turns ON the power supplies to the microscope, monitor, power supply unit, hard-copy unit and transmitted light unit (when the transmitted light detector is used).

- Turning the monitor ON

  (Refer to the instruction manual of your monitor.)

  As this unit should be connected to the power outlet unit, leave its power switch in the ON position.

- Turning the microscope ON

  (Refer to the instruction manual of the microscope combined with the system.)

  As this unit should be connected to the power outlet unit, leave its power switch in the ON position.

- Turning the transmitted light unit (when the transmitted light detector is used)

1-2-2 Starting the Software

NOTE **Before starting up this software, wait for more than 2 minutes after turning the microscope and power supply unit ON.**

TIP When executing a function that does not need image acquisition such as analysis of image data previously saved in a file, it is enough to turn only the computer and hard-copy unit ON.

NOTE **Before proceeding to the following, wait for more than 2 minutes after turning the microscope and power supply unit ON.**

NOTE **When using the AX70A and U-MCB, start the software while the MAIN screen of U-MCB is displayed.**

1. Input the user name and password, and log onto Windows NT.

NOTE **Input the user name authorized as an Administrator.**



FLUOVIEW icon

2. Double-click the [FLUOVIEW] icon on the desktop.

TIP When more than one user uses the FV500, each user shall log into it in different ways. See Appendix H, “User Registration of FV500” in this Volume.

TIP It takes 20 to 30 seconds to start up software since double-clicking the [FLUOVIEW] icon.

3. The following window appears when the FLUOVIEW software starts.

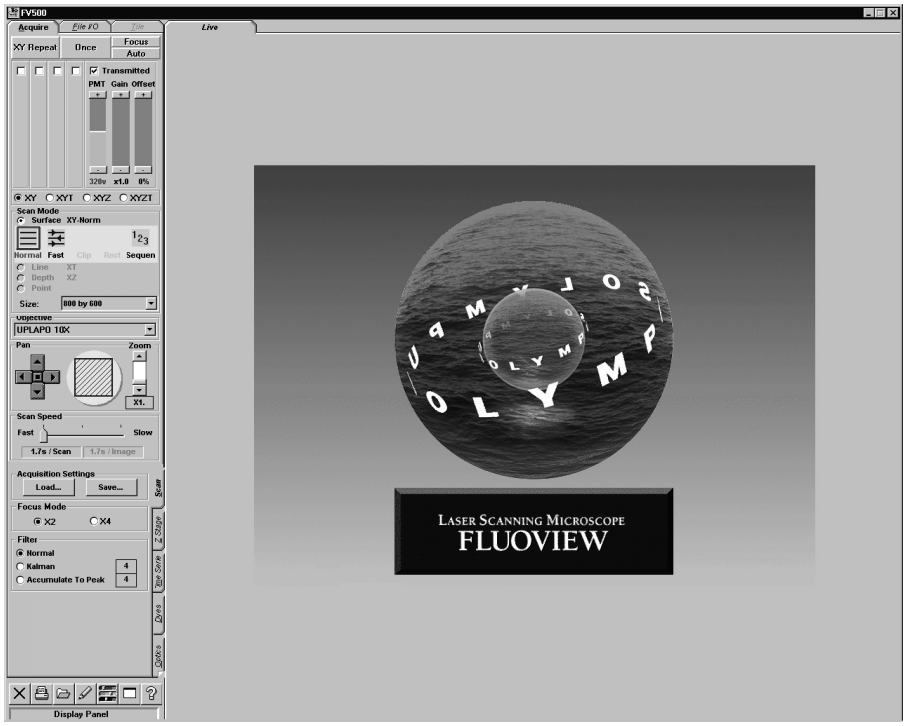


Fig. 1-3 Window at Start-up

1-2-3 Focusing on the Specimen

1-2-3-1 Combination with BX

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.

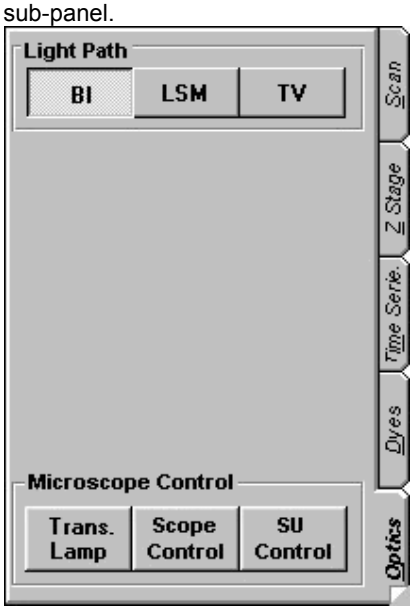
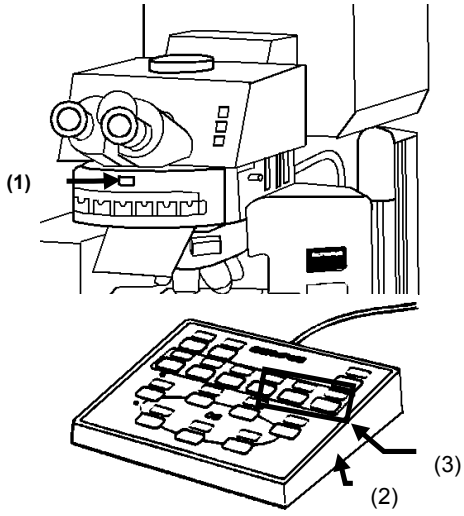


Fig. 1-4 [Optics] Sub-panel



2. Select the <BI> button in the [Light Path] group box.
   The <BI> button looks pushed in to indicate that it is selected.

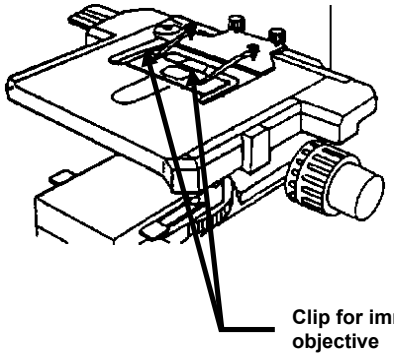
3. Push the cube button (3) of the hand switch (2) to engage the optimum cube for specimen dye. In the cube display window (1), the selected cube is displayed.

4. Focus on the specimen by looking into the eyepiece. Be sure to adjust the diopter of the eyepiece in advance. (Refer to the instruction manual of the BX microscope.)

When the Z motor is in use, clear the check mark in the [Locked] check box in the [Z Stage] sub-panel in the [Acquire] panel (see section 2-2-1-4-7 of this volume), then focus on the specimen by operating the fine focus adjustment knob of the microscope. Be sure not to operate the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z-Motor.

NOTE

The specimen may float during oil-immersed observation. In this case, prepare an optional clip for immersion objective and attach it as shown on the left.



Clip for immersion objective

If you want to use a differential interference unit in transmitted light observation, refer to the instruction manual of your microscope.

NOTE

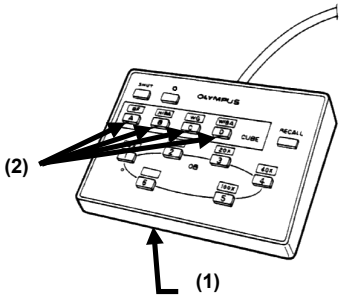
With transmitted light differential interference observation using an immersion objective, set the microscope's field diaphragm so that it circumscribes the field of view. Otherwise the contrast may become degrade.

1-2-3-2 Combination with AX

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.

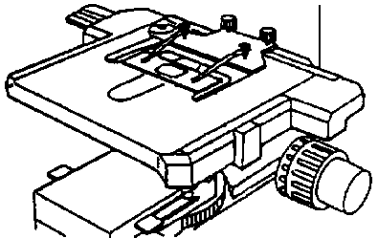


Fig. 1-5 [Optics] Sub-panel



2. Select the <BI> button in the [Light Path] group box.
   The <BI> button looks pushed in to indicate that it is selected.
3. Engage a cube in the light path by pressing the desired cube conversion button (2) on the hand switch for transmitted/reflected light (1).
4. Focus on the specimen by looking into the eyepiece. Be sure to adjust the diopter of the eyepiece in advance. (Refer to the instruction manual of the AX microscope.)

When the Z-motor is in use, clear the check mark in the [Locked] check box in the [Z Stage] sub-panel in the [Acquire] panel (see section 2-2-1-4-7 of this volume), then focus on the specimen by operating the fine focus adjustment knob of the microscope. Be sure not to operate the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z-Motor.



NOTE

The specimen may float during oil-immersed observation. In this case, prepare an optional clip for immersion objective and attach it as shown on the left.

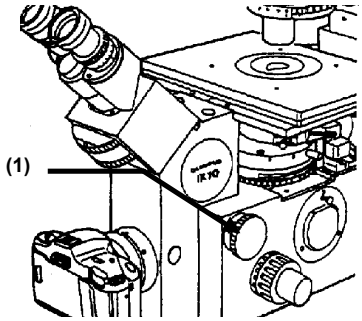


<TV> button

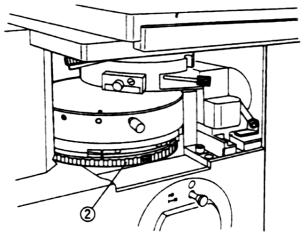
NOTE

To observe a TV image when the system is combined with a BX or AX microscope, click the <TV> button in the [Light Path] group box in the [Optics] sub-panel of the [Acquire] panel.

1-2-3-3 Combination with IX



1. Turn the light path selector (1) on the right side of the microscope to 

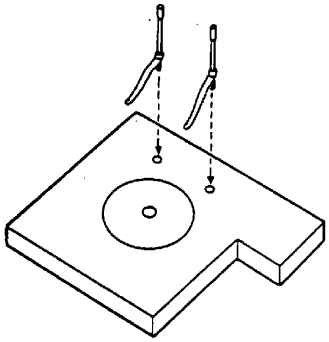


2. Engage the optimum cube for specimen dye by operating the cube turret (2).

3. Focus on the specimen by looking into the eyepiece.
Be sure to adjust the diopter of the eyepiece in advance. (Refer to the instruction manual of the IX50/70 microscope.)



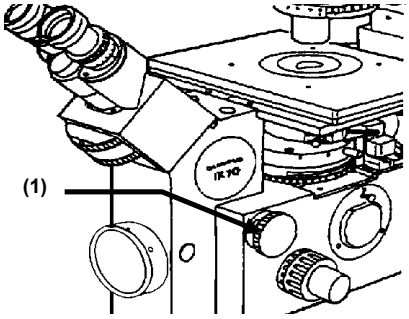
When the Z motor is in use, clear the check mark in the [Locked] check box in the [Z Stage] sub-panel in the [Acquire] panel (see section 2-2-1-4-7 of this volume), then focus on the specimen by operating the fine focus adjustment knob of the microscope. Be sure not to operate the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.



NOTE

The specimen may float during oil-immersed observation. In this case, prepare a stage clip (U-SCL) and attach it to the microscope as shown on the left.

1-2-3-4 Combination of IXBP (Bottom Port)



1. Turn the light path selector (1) on the right side of microscope to LSM.

2. From the index tabs on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.

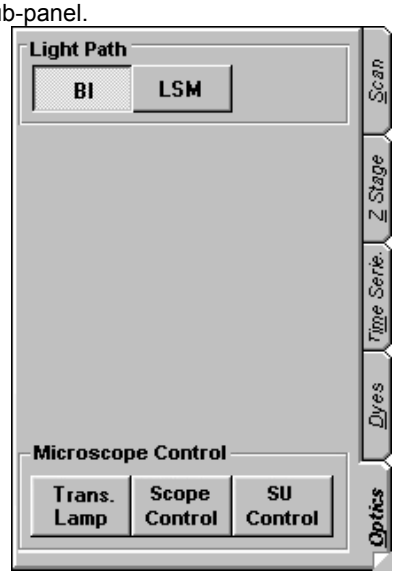
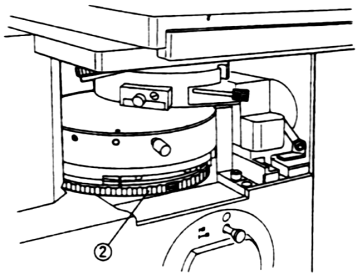


Fig. 1-4 [Optics] Sub-panel

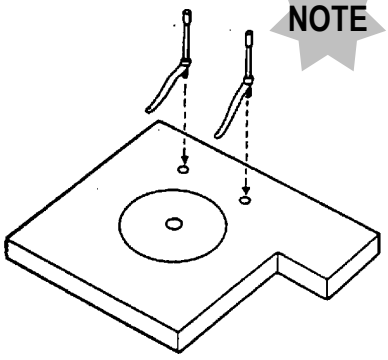
3. Click the <BI> button in the [Light Path] group box.
   The <BI> button looks pushed in to indicate that it is selected.



4. Rotate the cube turret (2) to engage the cube matching the specimen dye in the light path.

5. Look into the eyepiece and bring the specimen into focus.
   Be sure to adjust the eyepiece diopter correctly (refer to the instruction manual for the IX50/70).

When the Z-motor is in use, clear the check mark in the [Locked] check box in the [Z Stage] sub-panel in the [Acquire] panel (see section 2-2-1-4-7 of this volume) before bringing the specimen into focus using the focus adjustment knobs of the microscope. If the fine focus adjustment knob of the microscope is rotated while the [Locked] check box is checked, the Z-motor may be destroyed.



NOTE

The specimen may float during immersed observation. In this case, attach the stage clip (U-SCL) to the microscope.

1-2-4 Setting the LSM Light Path

1-2-4-1 Combination with Upright Microscope (BX)

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.

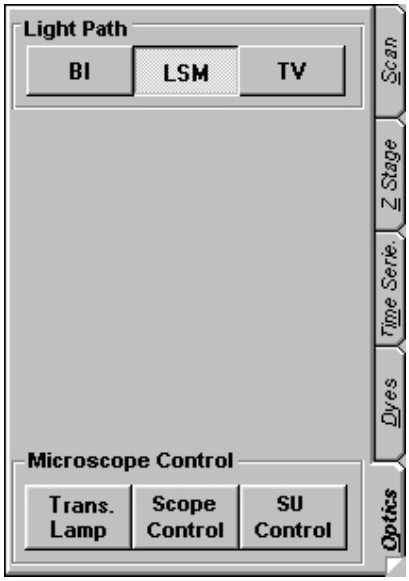


Fig. 1-6 [Optics] Sub-panel

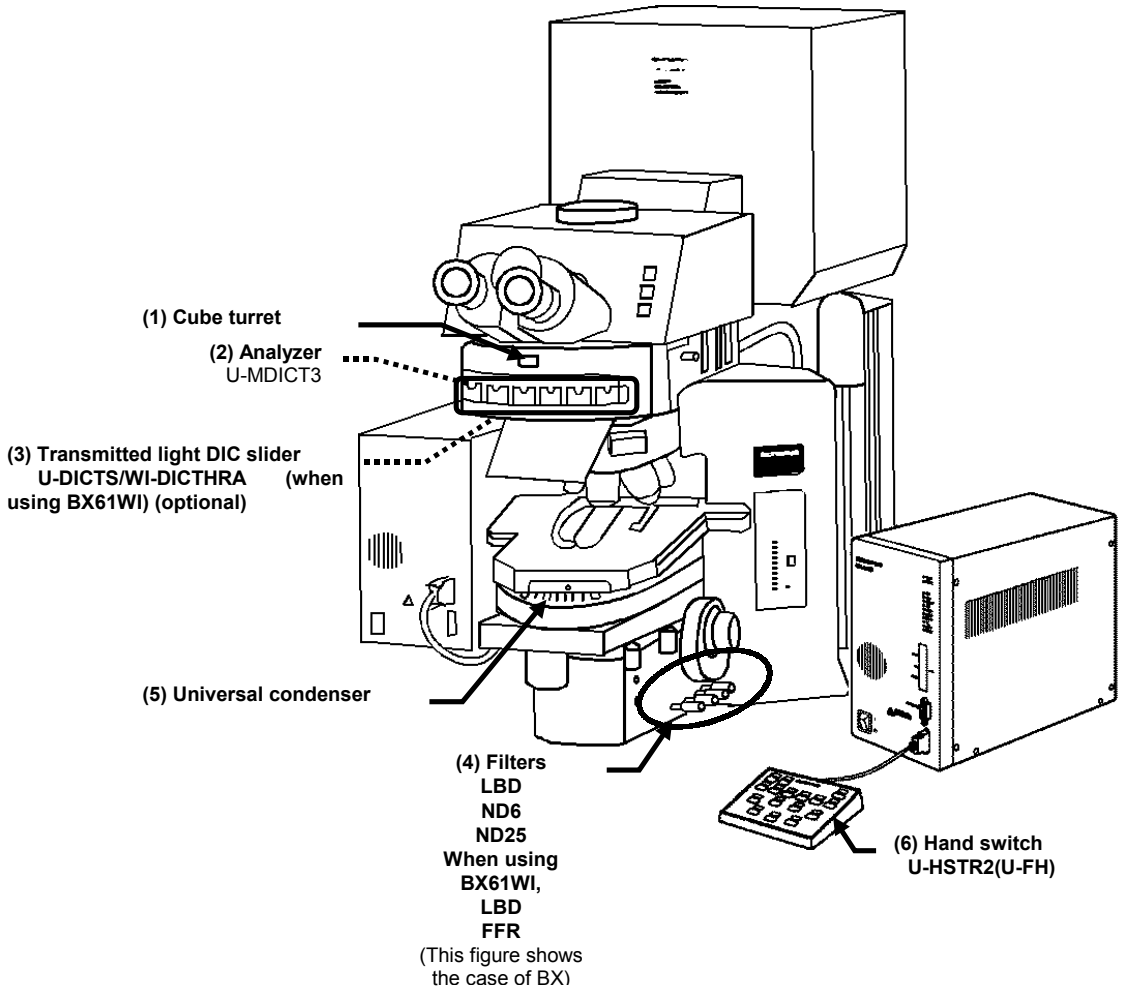
2. Select the <LSM> button in the [Light Path] group box.
   The <LSM> button looks pushed in to indicate that it is selected.
   (When scanning is started while the <BI> button is selected, the LSM light path is selected automatically. It is switched back to the visual observation automatically when scanning completes.)

3. Push the hand switch button to (8) set  to be displayed in the cube display window (1) on the reflected light fluorescence vertical illuminator

4. **When the U-MDICT3 analyzer (2) is in use, disengage it from the light path by setting the switch to the pulled-out position.**

5. When only fluorescence observation is required, disengage the U-DICTHR/WI-DICTHRA transmitted DIC (3) by setting the switch to the pulled-out position. When transmitted light differential interference observation or simultaneous fluorescence + transmitted light differential interference observation is required, engage the U-DICTHR/WI-DICTHRA and the optimum transmitted light DIC for the objective in the light path by operating the universal condenser (5).

6. For transmitted light observation, disengage any filter (4) from the light path.
When you perform transmitted observation using laser with BX61WI, use the filter knob to disengage the LBD from the light path and engage the FR (Frost) into the light path. Disengaging the FR (Frost) from the light path may generate interference fringes on an image.



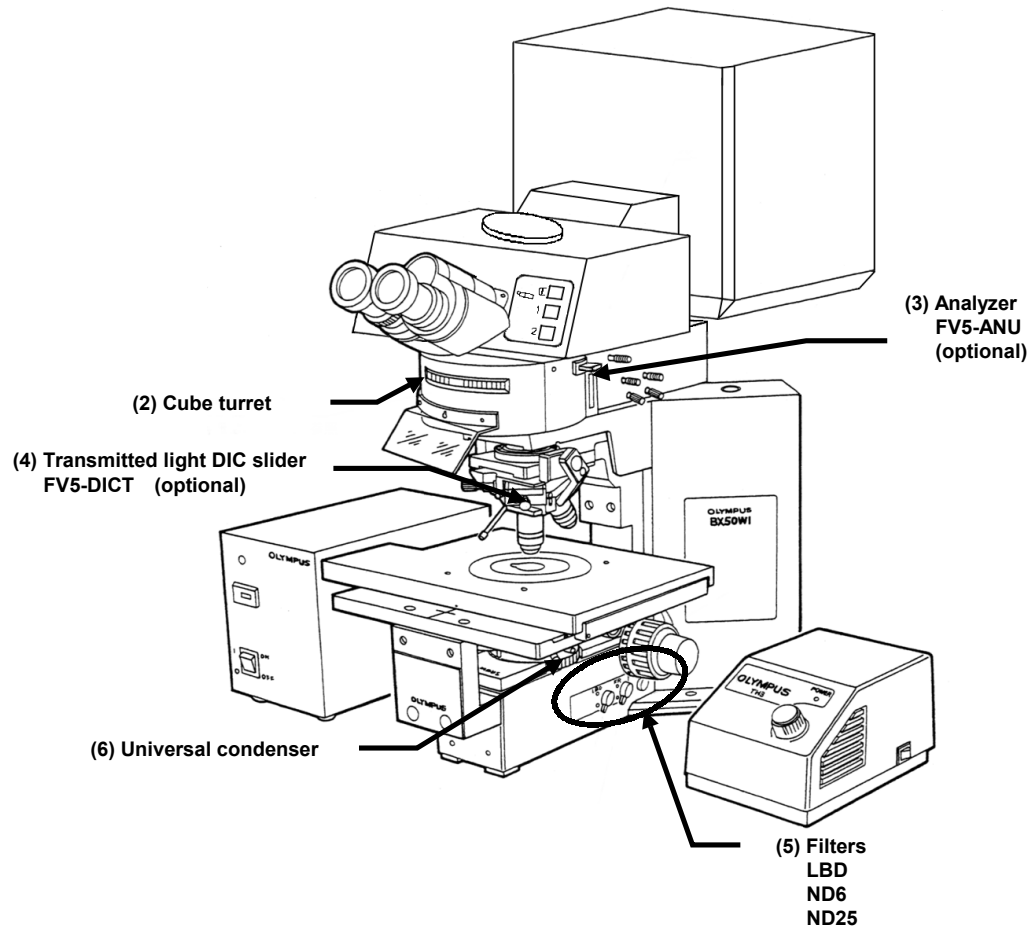
1-2-4-2 Combination with Inverted Microscope (BX50WI)

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.



Fig. 1-7 [Optics] Sub-panel

2. Select the <LSM> button in the [Light Path] group box.
   The <LSM> button looks pushed in to indicate that it is selected.
   (When scanning is started while the <BI> button is selected, the LSM light path is selected automatically. It is switched back to the visual observation automatically when scanning completes.)
3. Rotate the cube turret (2) of the universal vertical illuminator to ◯.
4. **When the FV5-ANU analyzer (3) is in use, disengage it from the light path by setting the switch to the pulled-out position.**
5. When only fluorescence observation is required, disengage the FV5-DICT transmitted light DIC slider (4) by setting the switch to the pulled-out position. When transmitted light differential interference observation or simultaneous fluorescence + transmitted light differential interference observation is required, engage the FV5-DICT and the optimum transmitted light DIC slider for the objective in the light path by operating the universal condenser (6).
6. For transmitted light observation, disengage the LBD filter (5) from the light path and engage the FR (frost) filter in the light path by operating the filter levers. If the FR filter is disengaged from the light path, the image may suffer from stripe interference.


(3) Analyzer
FV5-ANU
(optional)
(2) Cube turret
(4) Transmitted light DIC slider
FV5-DICT (optional)
(6) Universal condenser
(5) Filters
LBD
ND6
ND25
OLYMPUS
BX50WI

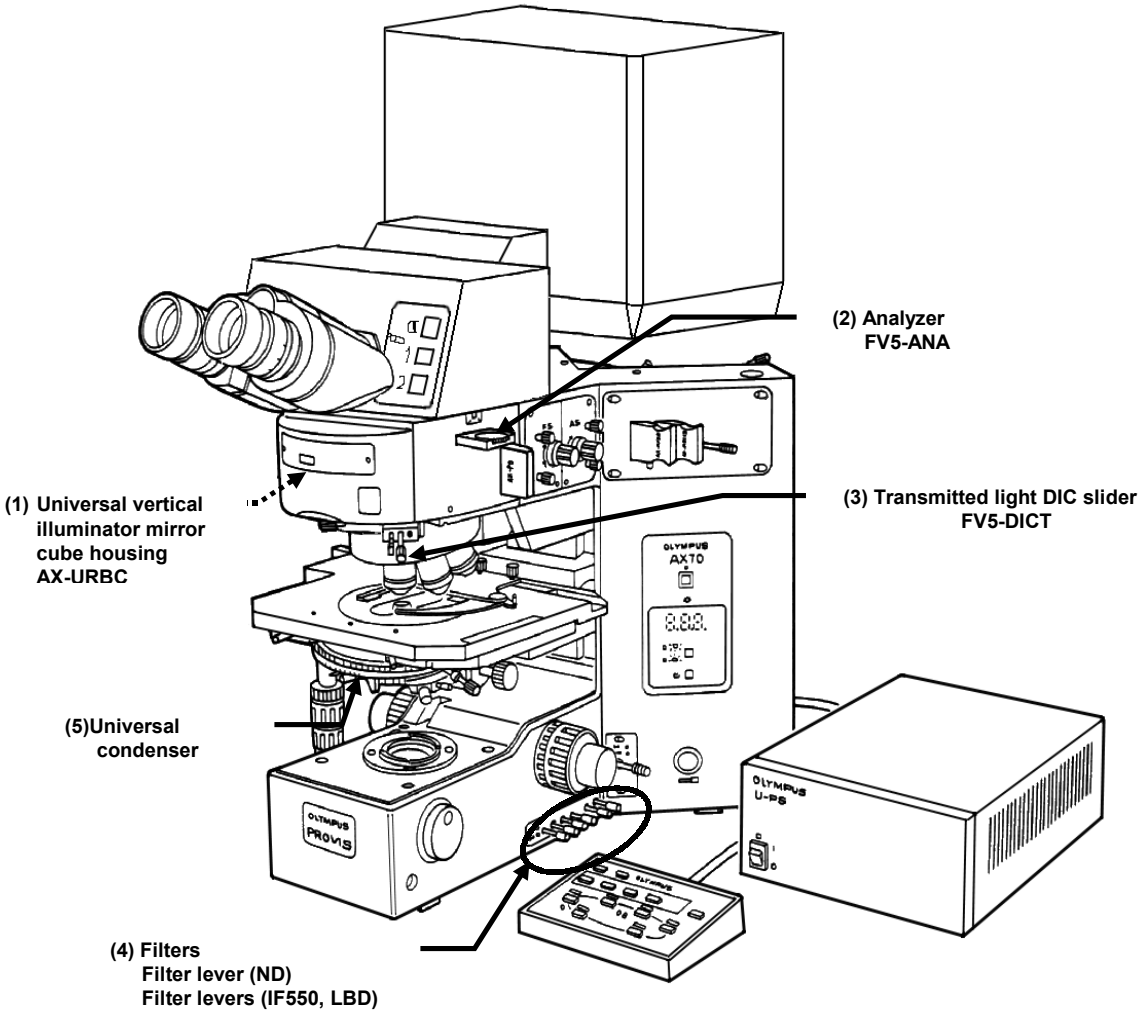
1-2-4-3 Combination with Upright Microscope (AX)

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.

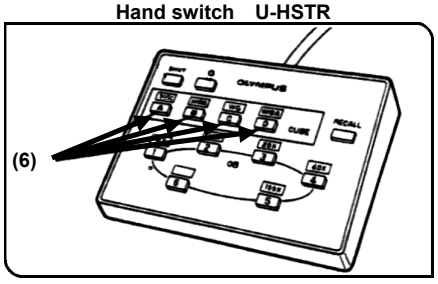


Fig. 1-8 [Optics] Sub-panel

2. Select the <LSM> button in the [Light Path] group box.
   The <LSM> button looks pushed in to indicate that it is selected.
   (When scanning is started while the <BI> button is selected, the LSM light path is selected automatically. It is switched back to the visual observation automatically when scanning completes.)
3. Set a cube in the Universal vertical illuminator mirror cube housing (1) by pressing the desired cube conversion button (6) on the U-SHTR hand switch.
4. **When the FV5-ANA analyzer (2) is in use, disengage it from the light path by setting the switch to the pulled-out position.**
5. When only fluorescence observation is required, disengage the FV5-DICT transmitted light DIC slider (3) by setting the switch to the pulled-out position. When transmitted light differential interference observation or simultaneous fluorescence + transmitted light differential interference observation is required, engage the FV5-DICT and the optimum transmitted light DIC slider for the objective in the light path by operating the universal condenser (5).
6. For transmitted light observation, disengage any filter (4) from the light path.



Hand switch U-HSTR



1-2-4-4 Combination with Upright Microscope (AX70A)

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.

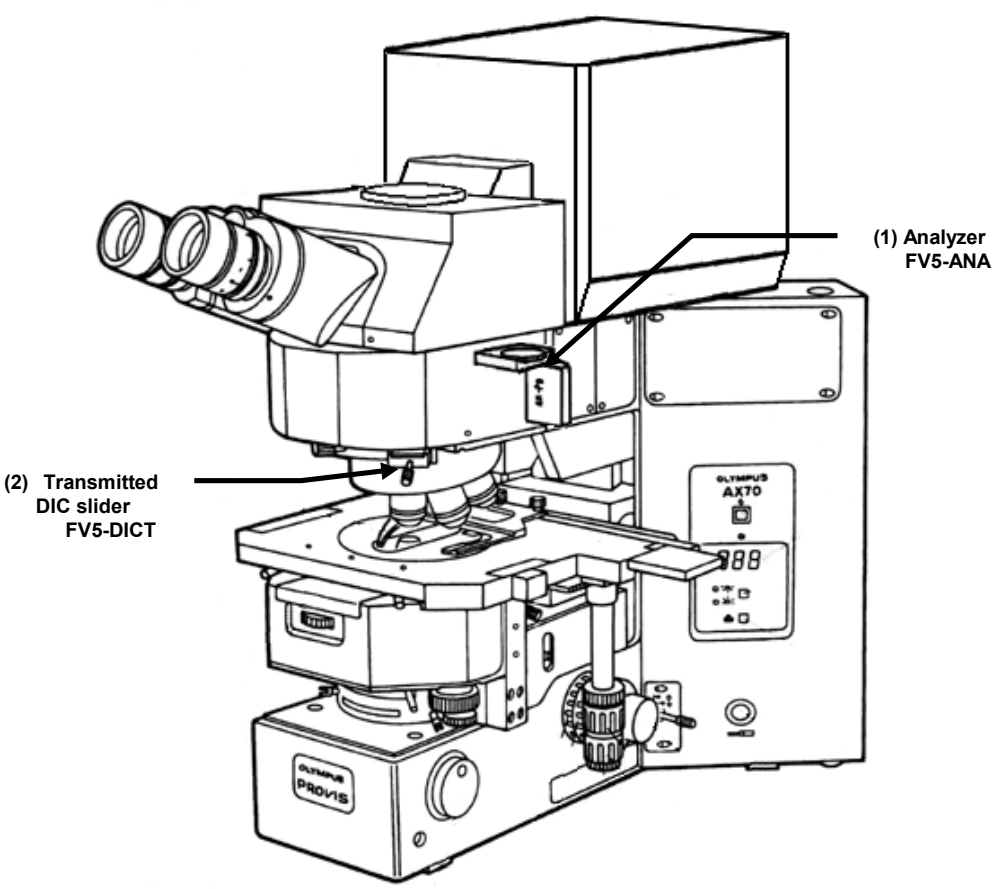


Fig. 1-9 [Optics] Sub-panel

2. Select the <LSM> button in the [Light Path] group box.
   The <LSM> button looks pushed in to indicate that it is selected.
   (When scanning is started while the <BI> button is selected, the LSM light path is selected automatically. It is switched back to the visual observation automatically when scanning completes.)
3. **When the FV5-ANA analyzer (1) is in use, disengage it from the light path by setting the switch to the pulled-out position.**
4. When only fluorescence observation is required, disengage the FV5-DICT transmitted light DIC slider from the light path by pulling it out.
   When the fluorescence + transmitted light simultaneous interference observation is required, the fluorescence resolution may be deteriorated slightly due to the engagement of the FC5-DICT in the light path.

TIP

The cube turret is set automatically to ○.
The optimum transmitted light DIC element for the objective in use is engaged automatically in the light path.

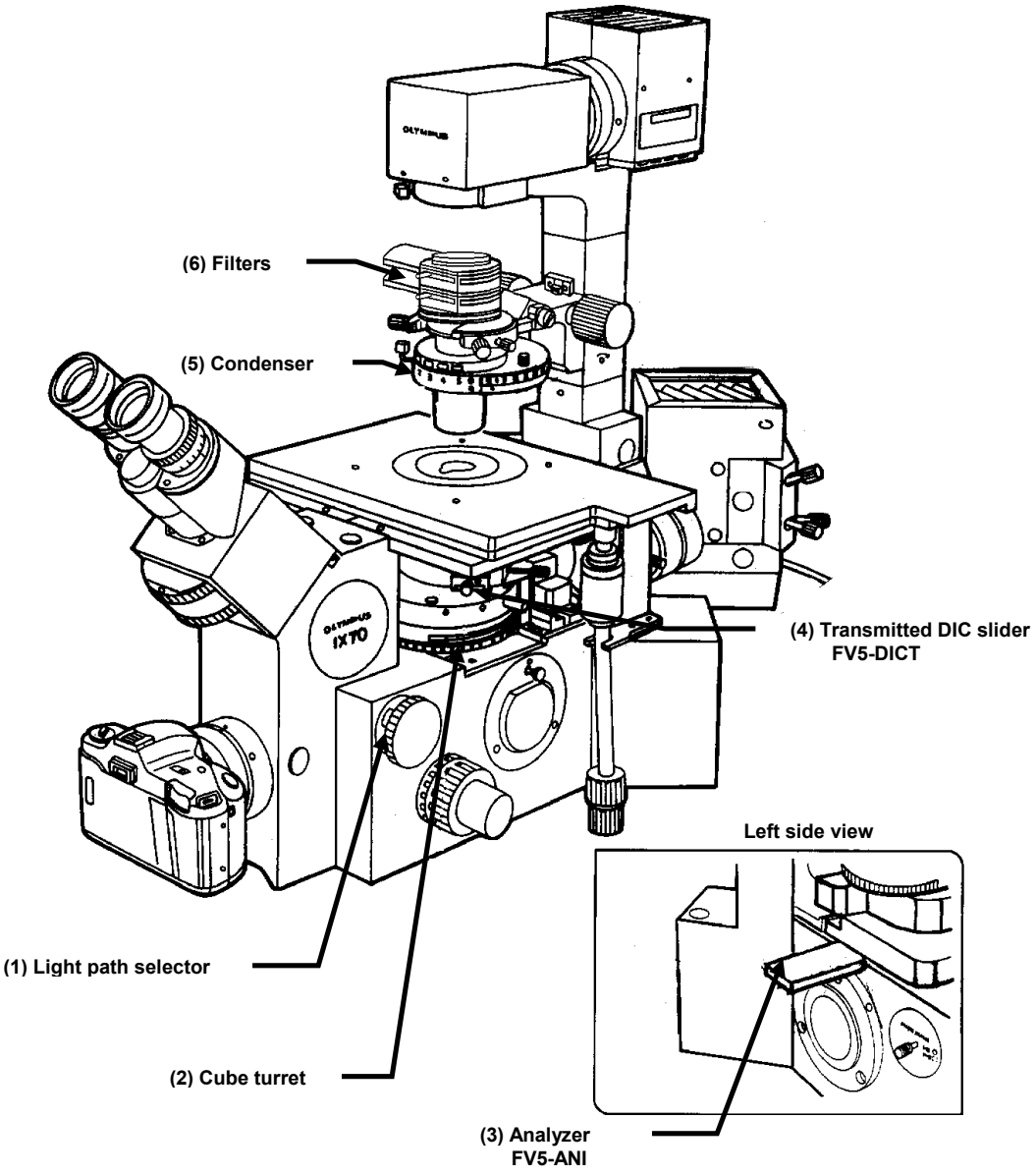

(1) Analyzer
FV5-ANA
(2) Transmitted
DIC slider
FV5-DICT
OLYMPUS
AX70
OLYMPUS
PROVIS

1-2-4-5 Combination with Inverted Microscope (IX)

1. Turn the light path selector (1) to SP.
2. Set the intermediate magnification knob (7) to 1X. (The 1.5X position cannot be used.)
3. Rotate the cube turret of the reflected light fluorescence unit to ○.
4. **When the FV5-ANI analyzer (3) is in use, disengage it from the light path by setting the switch to the pulled-out position.**
5. When only fluorescence observation is required, disengage the FV5-DICT transmitted DIC slider (4) by setting the switch to the pulled-out position. When transmitted light differential interference observation or simultaneous fluorescence + transmitted light differential interference observation is required, engage the FV5-DICT and the optimum transmitted light DIC slider for the objective in the light path by operating the universal condenser (5).

   With simultaneous fluorescence + transmitted light differential interference observation, leaving the FV5-DICT engaged in the light path will degrade the fluorescence image resolution somewhat.
6. For transmitted light observation, disengage filters (6) other than the FR (frost) filter from the light path.

   Be sure to engage the FR (frost) filter in the light path.


(6) Filters
(5) Condenser
(4) Transmitted DIC slider
FV5-DICT
Left side view
(1) Light path selector
(2) Cube turret
(3) Analyzer
FV5-ANI

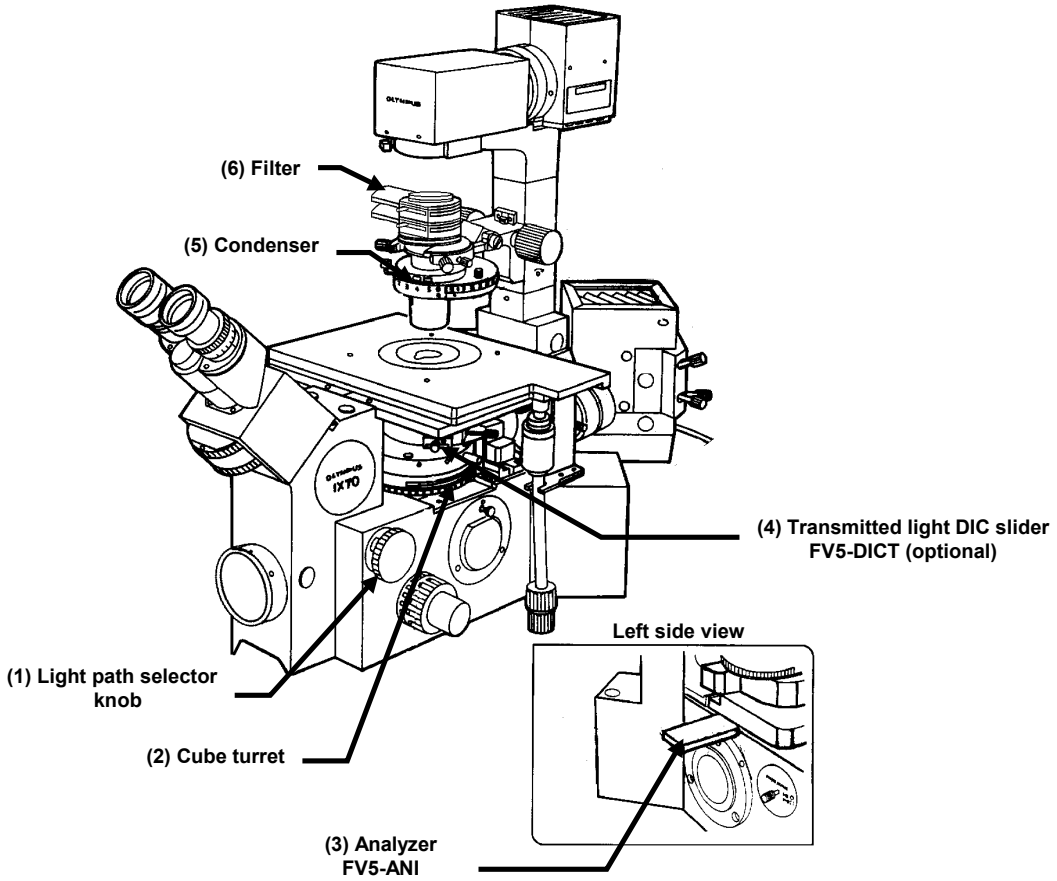
1-2-4-6 Combination of IXBP (Bottom Port)

1. From the index tabs on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.



Fig. 1-5 [Optics] Sub-panel

2. Click the <LSM> button in the [Light Path] group box.
   The <LSM> button looks pushed in to indicate that it is selected.
   (When scanning is started while the <BI> button is selected, the LSM light path is selected automatically. It is switched back to the visual observation automatically when scanning completes.)
3. Rotate the cube turret (2) of the vertical fluorescence illuminator to ◯.
4. **If the FV5-ANI analyzer (3) is engaged in the light path, pull the lever out to disengage it.**
5. When only fluorescence observation is required, disengage the FV5-DICT transmitted light DIC slider (4) from the light path. When performing transmitted DIC observation or fluorescence + transmitted light DIC simultaneous observation, engage the FV5-DICT as well as the transmitted light DIC slider matching the objective in use in the light path by operating the condenser (5).
   With the fluorescence + transmitted light DIC simultaneous observation, the presence of the FV5-DICT in the light path may deteriorate the fluorescence image resolution somewhat.
6. During transmitted light observation, be sure to disengage the filter (6) from the light path.


(6) Filter
(5) Condenser
(4) Transmitted light DIC slider
FV5-DICT (optional)
Left side view
(1) Light path selector
knob
(2) Cube turret
(3) Analyzer
FV5-ANI

1-2-5 Selecting the Dyeing Method

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

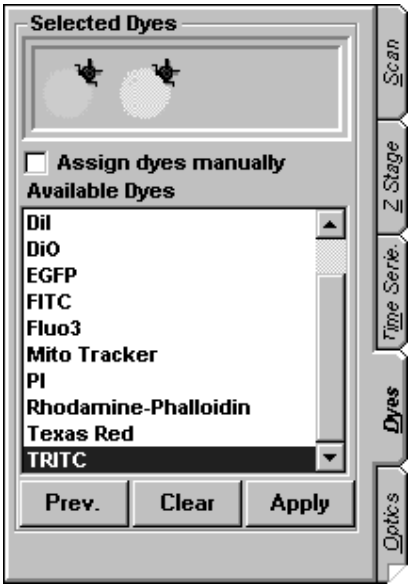


Fig. 1-10 [Dyes] Sub-panel

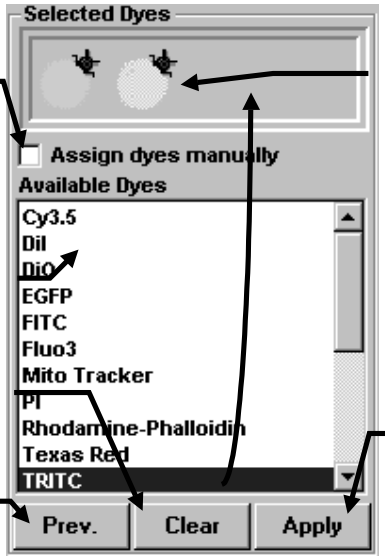
2. Select the specimen dyeing method by dragging [FITC] and [TRITC] in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

[Assign dyes manually] check box
Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

[Available Dyes] list box
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Clear> button
Clear the set dyeing method.

<Prev.> button
Sets the dyeing method which was set last time by clicking the <Apply> button.



Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

<Apply> button
Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.

3. Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

TIP

When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the switched filter. And the dyeing method is shown in the [Ch] group box.

The Confocal Aperture value is also set automatically according to the wavelength and the objective for every channel.

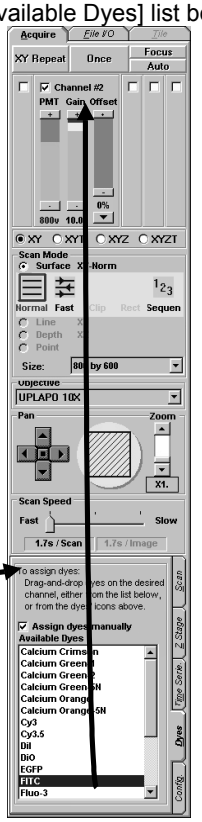
TIP

If you have changed the objective, click the <Apply> button in the [Dyes] sub panel. The Conforcal Aperture value is set appropriately.

One Point!

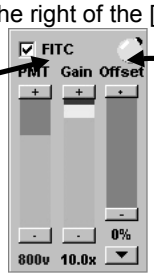
The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.



The dyeing method is set

Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

1-2-6 Selecting the Filters

The excitation filter, spectral filter and barrier filters are set automatically to the light path according to the dyeing method selected for the specimen.

To change filters, see section 1-3-2-4, “Configuring the Filters” in this volume and follow instructions in the [Optical System Configuration] window.

The following table shows the possible combinations of the barrier and excitation filters.

Laser Combination	Excitation Filter	Spectral Filter 1	Spectral Filter 2	Spectral Filter 3	Barrier Filter 1	Barrier Filter 2	Barrier Filter 3	Barrier Filter 4
Ar 488	DM351/488(Fix)			(1)Mirror (2)Glass (3)SDM560 (4) - (5) -			(1)BA505 (2)BA505-550 (3) - (4) - (5) - (6) - (7) -	(1)BA560 (2)BA610 (3) - (4) - (5) - (6) - (7) -
Ar,HeNe(G) 488,543	DM488/543(Fix9)			(1)Mirror (2)Glass (3)SDM560 (4) - (5) -			(1)BA505 (2)BA505-525 (3) - (4) - (5) - (6) - (7) -	(1)BA560 (2)BA610 (3) - (4) - (5) - (6) - (7) -
Ar,Kr 488,568	DM488/568(Fix)			(1)Mirror (2)Glass (3)SDM560 (4) - (5) -			(1)BA505 (2)BA505-550 (3) - (4) - (5) - (6) - (7) -	(1)BA585 (2)BA610 (3) - (4) - (5) - (6) - (7) -

Laser Combination	Excitation Filter	Spectral Filter 1	Spectral Filter 2	Spectral Filter 3	Barrier Filter 1	Barrier Filter 2	Barrier Filter 3	Barrier Filter 4
Ar,HeNe(G),HeNe(R) 488,543,633	(1)BS2/8 (2)DM351/488 (3)DM488/543 (4)DM488/543/633 (5) - (6) - (7) -		(1)Mirror (2)Glass (3)SDM560 (4) - (5) -	(1)Mirror (2)Glass (3)SDM630 (4) - (5) -		(1)BA505 (2)BA505-525 (3) - (4) - (5) - (6) - (7) -	(1)BA565 (2)BA610 (3)BA560-600 (4) - (5) - (6) - (7) -	(1)BA660 (2) - (3) - (4) - (5) - (6) - (7) -
Ar,Kr,HeNe(R) 488,568,633	(1)BS2/8 (2)DM351/488 (3)DM488/568 (4)DM488/568/633 (5) - (6) - (7) -		(1)Mirror (2)Glass (3)SDM560 (4) - (5) -	(1)Mirror (2)Glass (3)SDM630 (4) - (5) -		(1)BA505 (2)BA505-550 (3) - (4) - (5) - (6) - (7) -	(1)BA585 (2)BA610 (3)BA585-615 (4) - (5) - (6) - (7) -	(1)BA660 (2) - (3) - (4) - (5) - (6) - (7) -
UV,Ar,HeNe(G),HeNe(R) 351,488,543,633	(1)BS2/8 (2)DM351/488 (3)DM351/543 (4)DM351 (5)DM488/543 (6)DM488/543/633 (7) -	(1)Mirror (2)Glass (3)SDM490 (4) - (5) -	(1)Mirror (2)Glass (3)SDM560 (4) - (5) -	(1)Mirror (2)Glass (3)SDM630 (4) - (5) -	(1)BA400-470 (2) - (3) - (4) - (5) - (6) - (7) -	(1)BA505 (2)BA505-525 (3) - (4) - (5) - (6) - (7) -	(1)BA565 (2)BA610 (3)BA560-600 (4) - (5) - (6) - (7) -	(1)BA660 (2) - (3) - (4) - (5) - (6) - (7) -
UV,Ar,Kr,HeNe(R) 351,488,568,633	(1)BS2/8 (2)DM351/488 (3)DM351/568 (4)DM351 (5)DM488/568 (6)DM488/568/633 (7) -	(1)Mirror (2)Glass (3)SDM490 (4) - (5) -	(1)Mirror (2)Glass (3)SDM560 (4) - (5) -	(1)Mirror (2)Glass (3)SDM630 (4) - (5) -	(1)BA400-470 (2) - (3) - (4) - (5) - (6) - (7) -	(1)BA505 (2)BA505-525 (3) - (4) - (5) - (6) - (7) -	(1)BA585 (2)BA610 (3)BA585-615 (4) - (5) - (6) - (7) -	(1)BA660 (2) - (3) - (4) - (5) - (6) - (7) -

- :Invaild

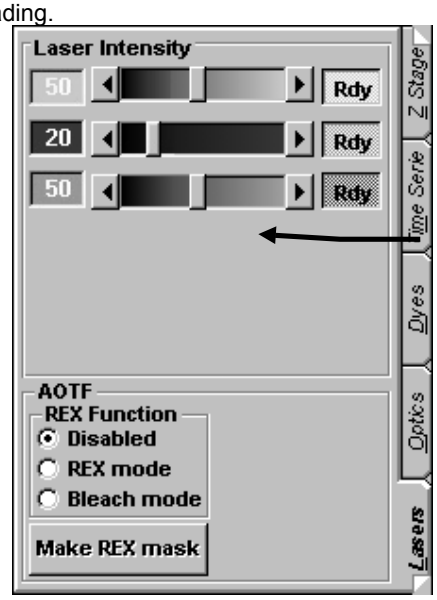
If the equipment of anoter filter set for laser configuration is required, please contact your local Olympus representative.

1-2-7 Selecting the ND Filters

- When the laser combiner is used

1. Display the [Acquire] panel.

   In the [Laser Intensity] group box of the [Lasers] sub-panel, set the ND filter scale of each laser according to the levels of specimen brightness, fluorescence crosstalk and fading.



[Laser Intensity] group box
Set the ND of the lasers in the scales.
The number of displayed lasers varies depending on the number of input channels.

Fig. 1-11 [Lasers] Sub-panel

When the Green HeNe laser is used, try ND of 50% by setting the [Intensity] scale in the [Lasers] group box to 50.

With other lasers, try ND of 10% first.

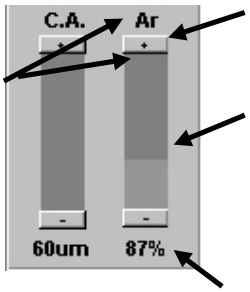
One Point!

After the dyeing method has been set with the [Dyes] sub-panel, the ND filters can be set using the [Ch] group box in the upper part of the [Acquire] panel.

1. In the upper part of the [Acquire] sub-panel, open the [Ch] group box for the ND to be changed.
2. Click the <More> button.

   The field as shown below is displayed below the [Ch] group box.

Display of the optimum laser and ND value which are set automatically according to the selected dyeing method.



Clicking this button allows fine adjustment of the ND value.

Clicking this field allows the ND value to be changed on a large scale.
The ND values which are usually used are displayed in green.

Display of the ND value set by clicking the <+> and <-> buttons or the field. The set ND value can also be changed by entering its value directly from the keyboard.

3. Vary the ND value using the Laser LED slider.

   Each click of the laser ND <+> or <-> varies the laser ND value by 1%.

   Each click of the laser ND slider varies the laser ND value by 5%.

1-2-8 Setting the Observation Condition

1-2-8-1 Setting the Objective Magnification

From the drop-down list on the center of the [Acquire] panel, select the objective being used with the microscope.



NOTE

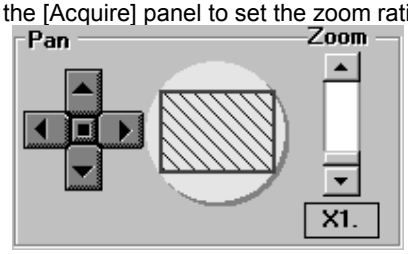
With a combination using a microscope other than the AX70A, the measurement results will be inaccurate if the objective magnification set here does not match the actual magnification of the objective in use.

TIP

If you change the objective, click the <Apply> button in the [Dyes] sub panel. The Conforcal Aperture value is set appropriately.

1-2-8-2 Setting the Zoom Ratio to 1X

Use the [Zoom] scale in the [Acquire] panel to set the zoom ratio to “X1”.

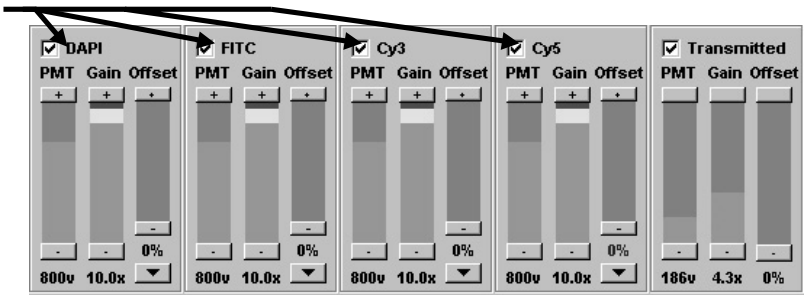


Using the UV-Ar laser, set the zoom ratio to “X2”.

1-2-8-3 Setting the Channels

1. In the [Ch] group box, make sure that the check boxes showing the applicable dyeing methods are check-marked to indicate that the channels are ready for image acquisition.
   If the check box of a channel is not check-marked, check it to make the channel ready.

Channel check boxes, with which dyeing methods are displayed



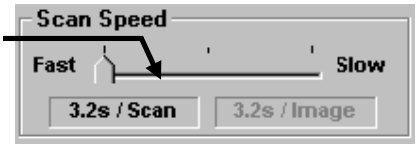
TIP

To display the information on all channels simultaneously, right-click the boundary between channel display boxes.
Click the boundary again to return to the original display.

1-2-8-4 Setting the Highest Scan Speed

1. Set the scan speed to the fastest speed by using the scale in the [Scan Speed] group box in the [Acquire] panel

[Scan Speed] group box
Set the scan speed by clicking a point on the scale line.



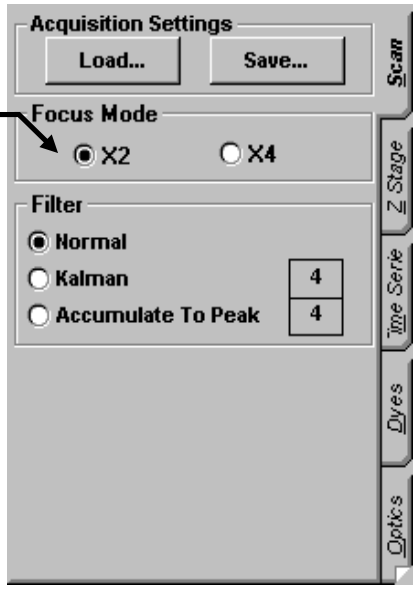
TIP

The focus mode makes it possible to increase the line skipped scan speed.

From the page tabs on the bottom right of the [Acquire] panel, select the [Scan] sub-panel.

Select either option button in the [Focus Mode] group box.

[Focus Mode] group box
[X2] option button
Acquires image at twice the highest speed.
[X4] option button
Acquires image at 4 times the highest speed.
Increasing the number of divided images in the [Display] panel, line skipped scan at 4 times (Focus) cannot be done.



The focus mode is enabled when acquiring images using the <Focus> button.

NOTE

The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.

1-2-8-5 Setting the XY Observation Mode

1. In the [Scan Mode] group box in the [Acquire] panel, select the [Surface] option button.
2. In the [Scan Mode] group box in the [Acquire] panel, select [800 by 600] from the [Size] drop-down list.
3. In the [Acquire] panel, select the XY observation mode option button.

1-2-8-6 Repeated Scanning Operation



<XY Repeat> button



<Focus> button

1. Select the <XY Repeat> button. The acquired image will be displayed in the [Live] panel.

TIP

Use the <FOCUS> button to acquire image at an even higher speed. If the specimen is already being scanned, stop scanning with the <STOP SCAN> button before selecting the <XY Repeat> button.

NOTE

The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.

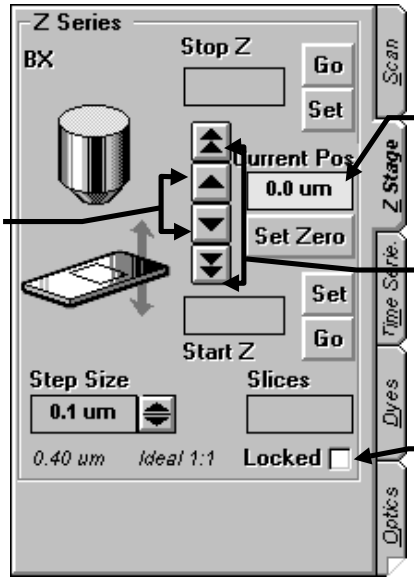
NOTE

Do not move FLOUVIEW FV500 Menu while acquiring an image.

1-2-8-7 Setting the Cross-section to be Observed

While acquiring image, move the Z stage to select the cross-section to be observed.

1. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.



<Z stage fine adjustment> buttons
Displace the Z stage by 0.1 μm per step.

[Current Pos] text box
Shows the current position of the stage. The value can also be entered directly from the keyboard.

<Z stage coarse adjustment> buttons
Displace the Z stage by 1.0 μm per step.

[Locked] check box
Enables the Z motor operation.

Fig. 1-12 [Z Stage] Sub-panel

2. Check the [Locked] check box in the [Z Stage] sub-panel.

Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

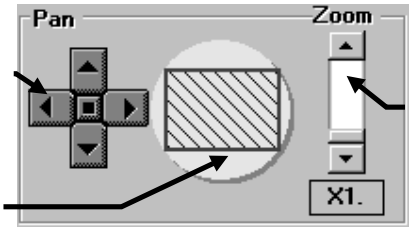
3. While observing the image in the [Live] panel, locate the plane to be observed by displacing the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

1-2-8-8 Setting the Area to be Observed

When the observation targets are concentrated in a narrow area or when observation of a specific area detail is required, the image of a limited area can be selected.

The 4 buttons represent directions, and clicking a button moves the acquired image area in the direction indicated by the button.
Clicking the square button on the center returns the acquired image area to the center.

Click a point inside the circle to change the position of the acquired image area directly.



Clicking a point in the scale area to change the value on a large scale.
Clicking the top or bottom arrow button allows fine adjustment of the value.
Dragging the square knob allows the value to be changed directly.

Fig. 1-13 [Pan]/[Zoom] Group Box

1-2-8-9 Setting a Lower Scan Speed

The scan speed can be decreased using the scale in the [Scan Speed] group box on the [Acquire] panel.

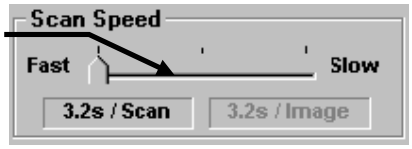
TIP

In general, setting a lower scan speed allows the acquired image quality to be improved.
However, a low scan speed also lengthens the time required for image acquisition.

NOTE

When the scan speed is decreased during fluorescence observation, the saturation of fluorescence may darken the image of certain types of specimens. In this case, increase the scan speed and increase the PMT Voltage or use accumulation in scanning.

[Scan Speed] group box
Set the scan speed by clicking a point on the scale line.



1-2-8-10 Stopping Repeated Scanning

After the brightness and gain have been adjusted, select the <STOP SCAN> button in the [Acquire] panel to stop scanning temporarily.



1-2-9 Acquiring Image



<Once> button

Select the <Once> button. The acquired image will be displayed in the [Live] panel.

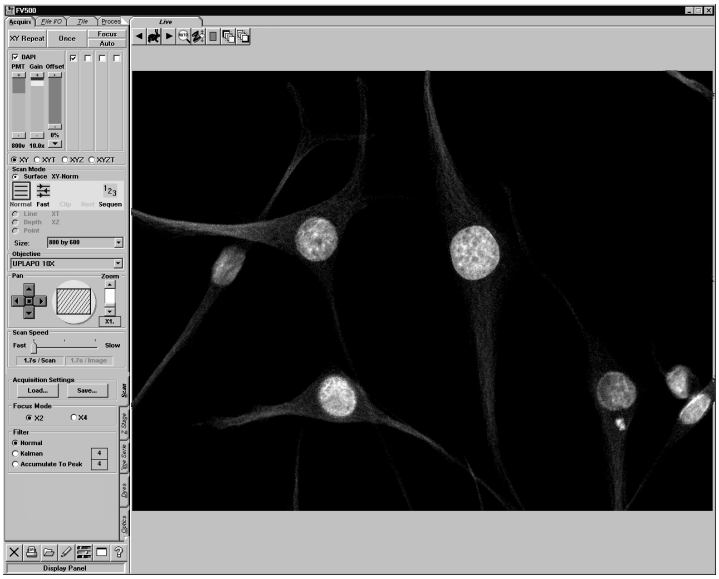
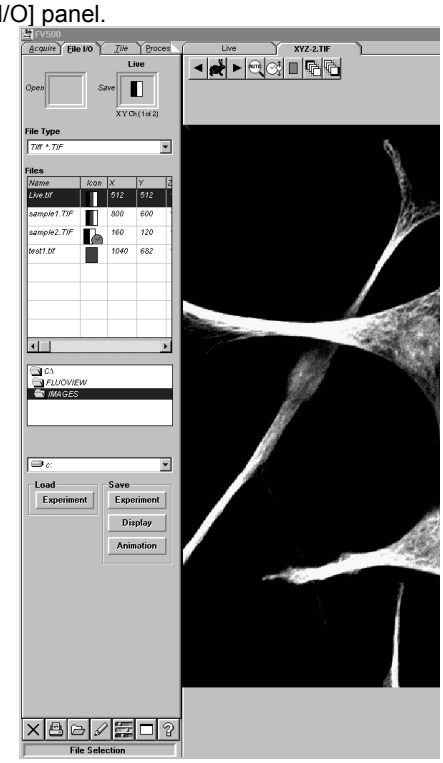


Fig. 1-14 Image Acquired in the [Live] Panel

1-2-10 Saving Image

1. Display the [File I/O] panel.





<Display channel switch> buttons

2. When saving images acquired with more than one channel, it is possible to select whether images from more than one image are saved simultaneously or only one of the images is saved.
   Use the <Display channel switch> buttons to select the images to be saved. The selected images will be saved under the conditions set for each channel.
   Example)When only the image of Channel 1 is displayed, only the image of Channel 1 will be saved.
3. Click <Experiment> button in the [Save] group box. The [Save Experiment As] dialog box will open.

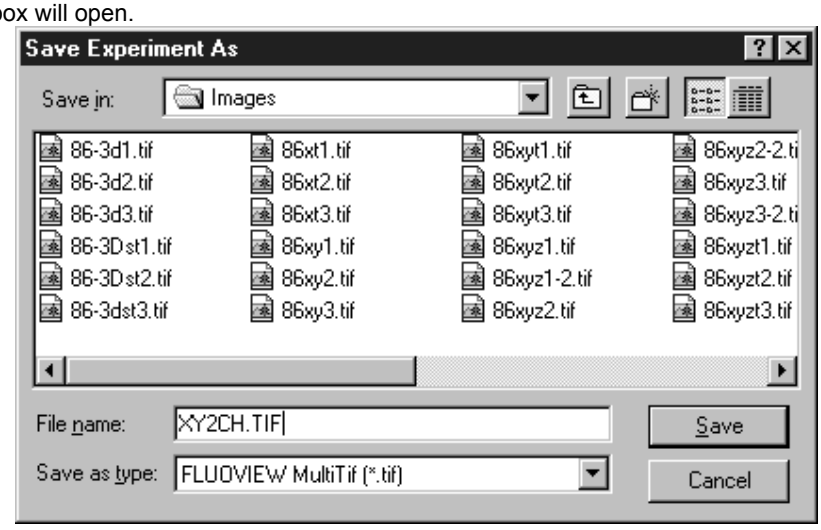


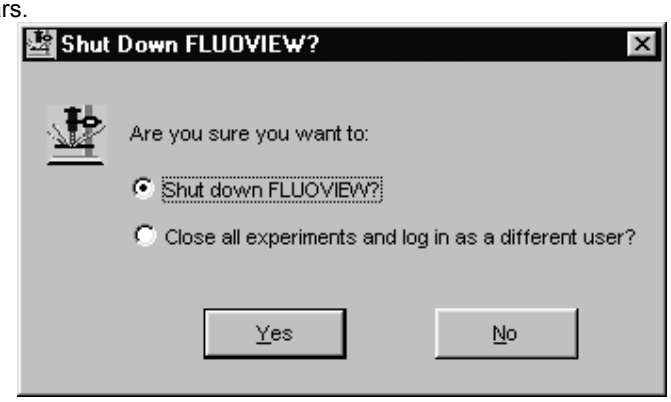
Fig. 1-15 [Save Experiment As] Dialog Box

4. Enter the file name in the [File name:] text box.
5. Select “FLUOVIEW MultiTif” from [Save as type:]
6. Click the <Save> button.

1-2-11 Exiting from the Software


<Exit> button

1. Click the <Exit> button in the toolbar at the bottom of the screen. This will allows you to exit the software. The [Shut Down FLUOVIEW?] dialog box as shown below appears.



2. To exit from this software, select the [Shut Down FLUOVIEW?] option button and click the <OK> button. Then this software finishes.

3. To logout from this software, select the [Close all experiments and log in as a different user?] option button and click the <Yes> button. The dialog box asking whether to save the observation data or not appears. If you want to save the data, click the <Yes> button, or the <No> button if saving is unnecessary.
   It returns to the FLUOVIEW login screen.

4. To shut down Windows, select the Windows <Start> button to display the [Start] menu, and select the [Shut Down] command from the menu.

5. When the [Shut Down Windows] dialog box appears, select the [Shut down the Computer?] option box and select the <Yes> button.
   Wait until message “It is now safe to turn off your Computer” is displayed

1-2-12 Turning Power Off

1. Turn off the power supply to the units (reflected light power supply, switched power outlet unit).

2. Turn the laser power OFF.
   - Ar laser:
     Turn the key to the OFF position, then set the power switch to OFF.
     (For details, refer to the instruction manual of your laser unit.)

   - Kr laser:
     Turn the key to the OFF position and wait for the fan to stop automatically when the laser unit has cooled down. Then set the power switch to OFF.
     (Refer to the instruction manual of your laser unit.)

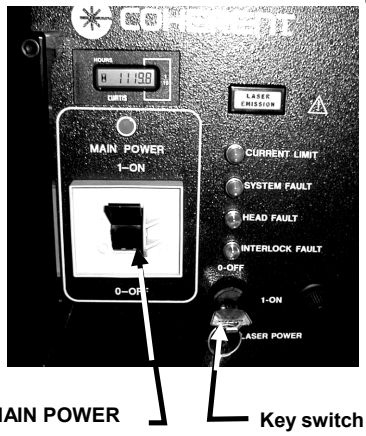
   - HeNe (Green / Red) laser:
     Turn the key to the OFF position.

   - UV-Ar laser:

1.1 Turn the key switch on the UV laser power supply to the “O-OFF” position.

1.2 Set the MAIN POWER lever on the UV laser power supply to the “O-OFF”.

UV laser power supply



TIP

Chiller is switched to auto cooling operation by interlocking. When it is left in auto cooling, it will automatically turn off in about 15 minutes.

While the heat exchanger is running, do not shut off the power supply to the power outlet where the chiller is connected.
If the power supply is turning on, the UV Ar laser tube may be destroyed by high temperature.
After the heat exchanger has turned off, leave it for more than an hour until the laser tube has cooled down before turning it on again.

- IR laser:

  Refer to the instruction manual of the IR laser (manufactured by MELLES GRIOT).

- HeCd laser:

  1. Turn the <OFF> button of remote interface module OFF.
     Wait till “LASER OFF” message appears on digital display. (For about 5 minutes to cool it down.)
  2. Turn the key switch of remote interface module to the OFF position.
  3. Turn the key switch of laser controller to the OFF position.
  4. Turn the rocker switch of laser controller to “0” (OFF).



Turning the laser power off by force may damage the laser tube.
Never omit the step 1 (the process for cooling down) except for emergency.

1-3 Online Help

The FLUOVIEW application comes with two kinds of online help facility:

- function help for referencing the function and operation procedure description while controlling the application, and
- microscope help providing information on the system setup.

1-3-1 Function Help

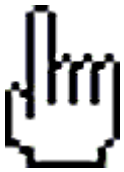
This section describes a simple method for displaying and consulting the online help on the functions and operation procedures.



<Help> button

The figure below shows the initial display (table of contents) of the FLUOVIEW Online Help window. To display this window, click the <Help> button in the toolbar at the bottom left of the screen.

Some words in the displayed information are shown in enhanced display (underscored or colored green). Clicking one of these words allows you to "jump" to the meaning of the word or to more information about its meaning.



Finger pointer

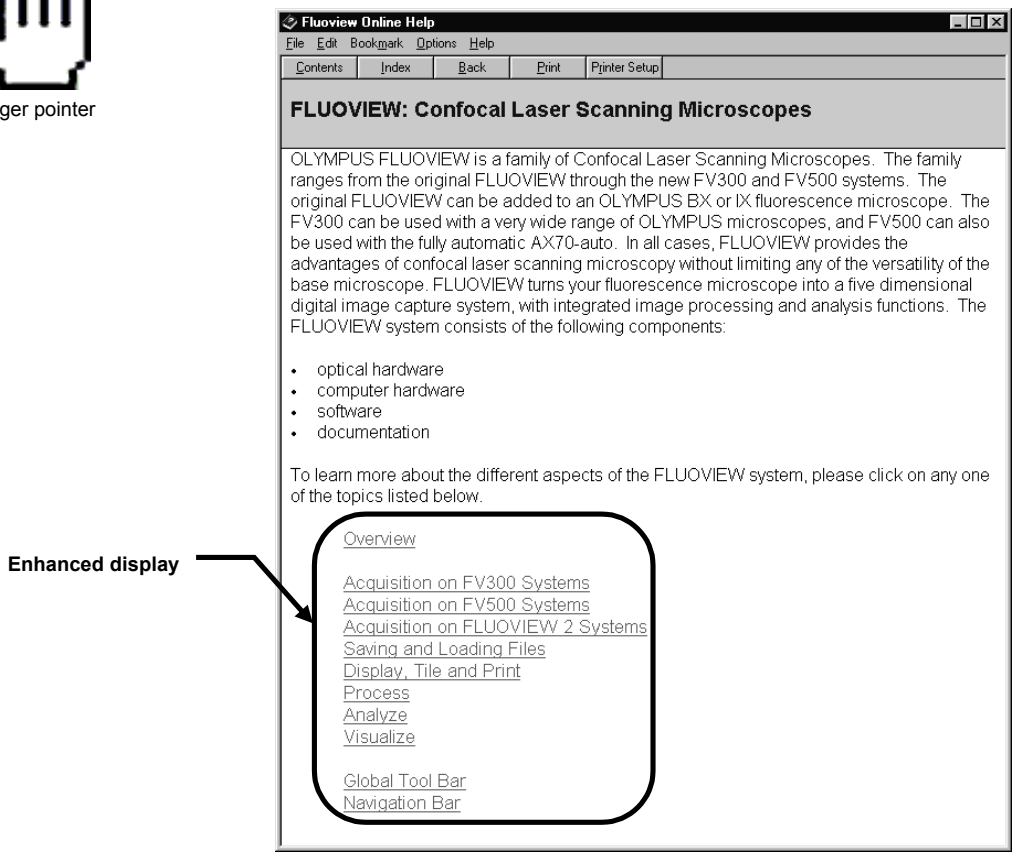


Fig. 1-16 Initial Window

TIP When the mouse pointer is placed on a word in enhanced display, the mouse pointer turns into a “finger pointer”.

One Point!

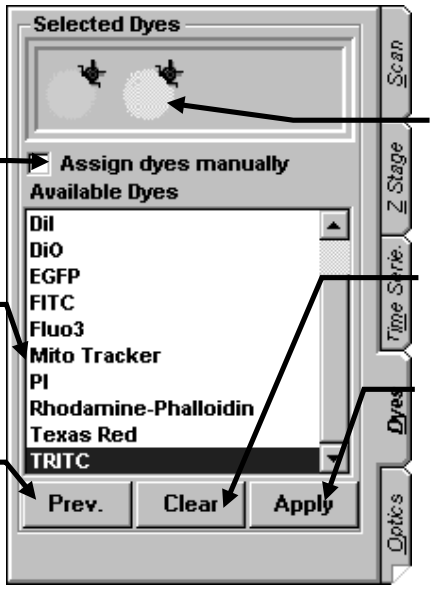
Click the <Contents> button to the initial display. Click the <Back> button to return to the previous information page.

1-3-2 Microscope Help

The microscope, scan unit and laser types can be set up from the FLUOVIEW software, by selecting the dyeing method and following the displayed guidance information.

- Selecting the Dyeing Method

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.



[Assign dyes manually] check box
Checking this enables the manual setting. Dragging the dyeing method directly to the [Ch] group box assigns the dyes to the desired channel.

[Available Dyes] list box
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Prev.> button
Sets the dyeing method which was set last time by clicking the <Apply> button.

Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

<Clear> button
Clear the set dyeing method.

<Apply> button
Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.

Fig. 1-17 [Dyes] Sub-panel

2. Select the specimen dyeing method by dragging desired dye names in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

3. Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

TIP

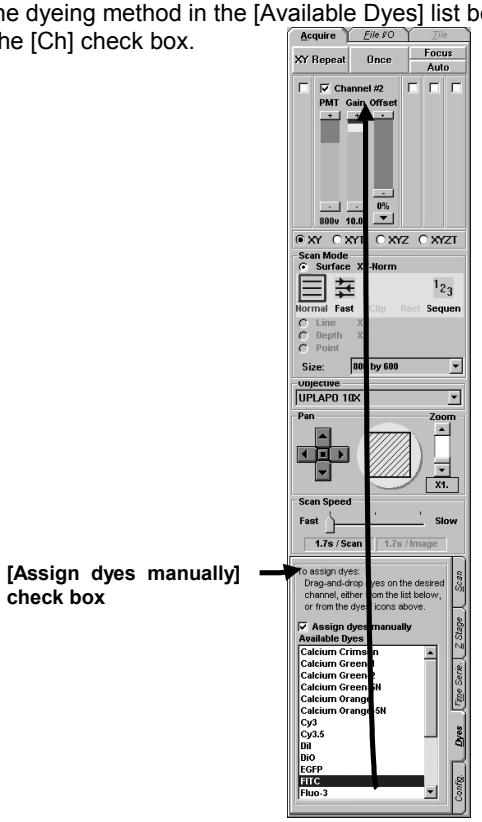
When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the changed filter. And the dyeing method is shown in the [Ch] group box.

The Confocal Aperture value is also set automatically according to the wavelength and the objective per channels.

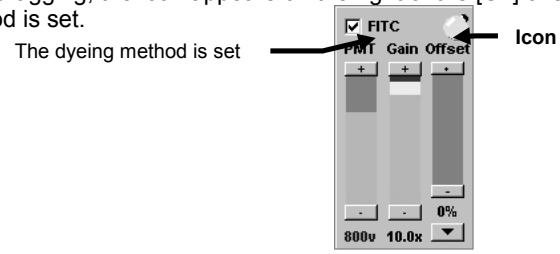
One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



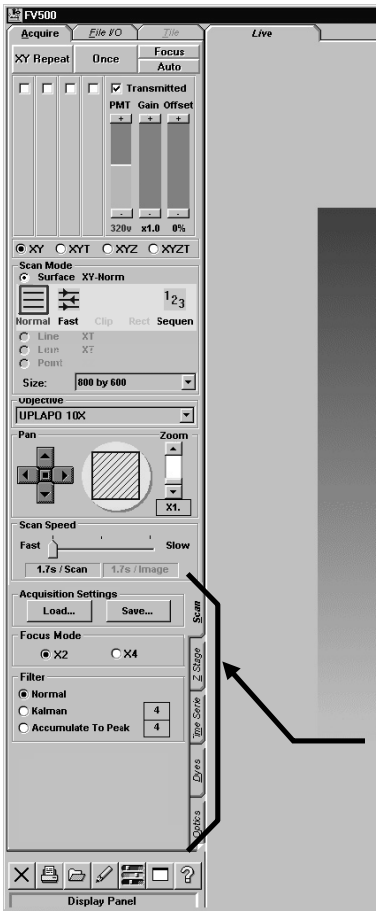
3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.



Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

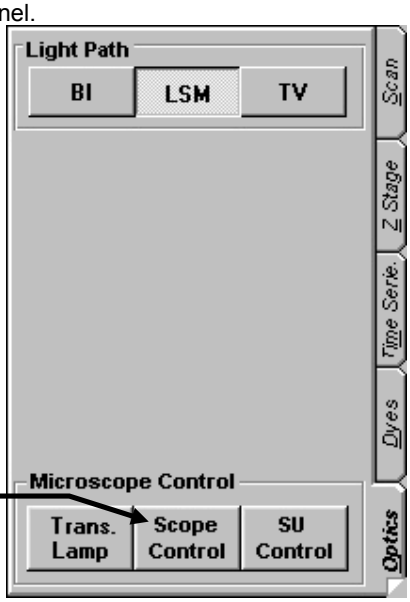
1-3-2-1 Configuring the Microscope

1. Display the [Acquire] panel.



[Scan]/[Z Stage]/[Time Series]/[Optics]/[Lasers] sub-panels
These are used to set the information required for image acquisition.

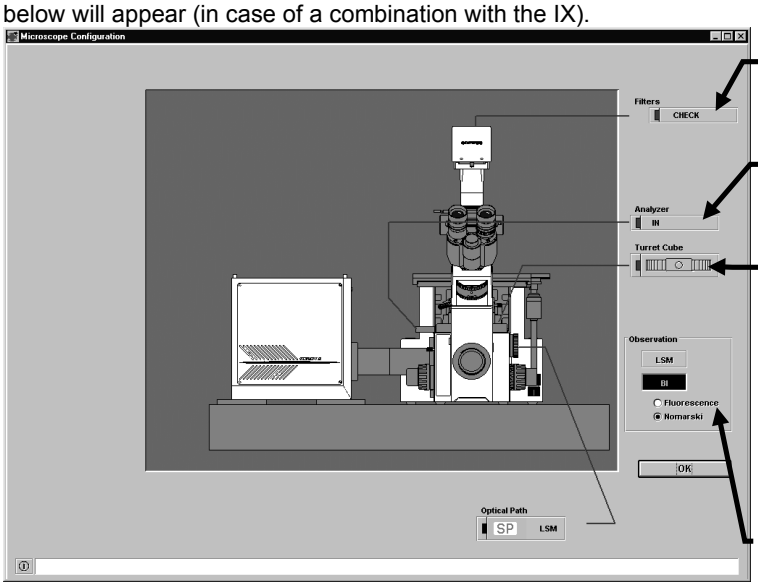
2. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.



<Scope Control> button
Displays useful information for the system setup.

Fig. 1-18 [Optics] Sub-panel

Select the <Scope Control> button at the bottom of the panel. The window as shown below will appear (in case of a combination with the IX).



[Filters] box
This represents the filters.

[Analyzer] box
Represents the FV5-ANA analyzer (optional).

[Turret Cube] box
Represents the cube turret.

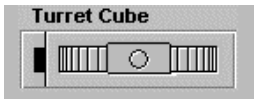
[Observation] group box
[LSM] represents the laser scanning microscopy light path, and [BI] represents the visual observation light path. [Fluorescence] represents fluorescence observation for both LSM and BI observations, and [Nomarski] represents transmitted light observation.

Fig. 1-19 [Microscope Configuration] Window



<BI> button

3. Select the <BI> button in the [Observation] group box, and select the microscopy from the option buttons below it. The points where system setting is to be changed for microscope observation will blink in red.



4. Change the system configuration (setup of cube turret, etc.) by following the guidance given by the red blinking light.
(For the operation of the lever, see section 1-1-1, “Microscope” in this volume.)

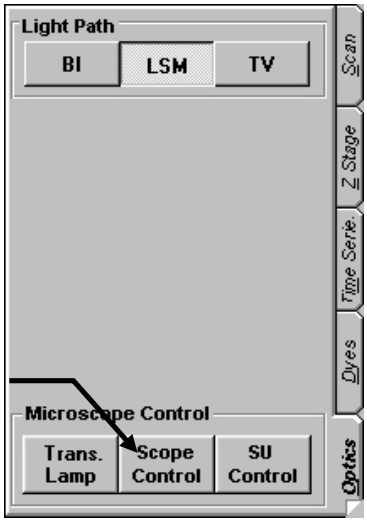
NOTE **The [Microscope Configuration] window is designed to give guidance on system configuration. The red blinking light does not stop even after the indicated configuration point has been changed.**

5. While looking into the microscope, move the stage and check the observed image.

1-3-2-2 Configuring the Microscope (Combination with BX51, BX61)

When the combination with BX51 or BX61 is in use, the microscope and scan unit (FV500) can be configured on the FLUOVIEW software.
Use the following procedure to configure the microscope.

1. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.

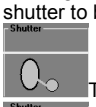


<Scope Control> button
Sets the system.

Fig 1-6 [Optics] Sub-panel

2. Select the <Scope Control> button on the bottom of the [Optics] sub-panel. The [BX Control] window as shown below appears.

[Shutter] group box
Clicking inside the box switches the EPI shutter to be closed/opened,



The shutter is opened.



The shutter is closed.

[EPI lamp]
Indicates the EPI lamp.

[Filter Turret] group box
Clicking the filter for reflected light observation to be set switches the turret automatically.

[LMT] group box
Selects the light path. <BI> is for direct observation, <LSM> is for LSM observation and <TV> is for TV observation.

[Filter Turret] group box
Clicking the filter for visual observation to be set switches the turret automatically.

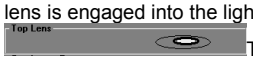
[Mirror Unit] group box
Clicking the cube automatically switches the turret.

[Nosepiece] group box
Click to change the objective.

[Top Lens] group box
Clicking inside the box switches the top lens to be engaged into the light path.



The top lens is engaged into the light oath.

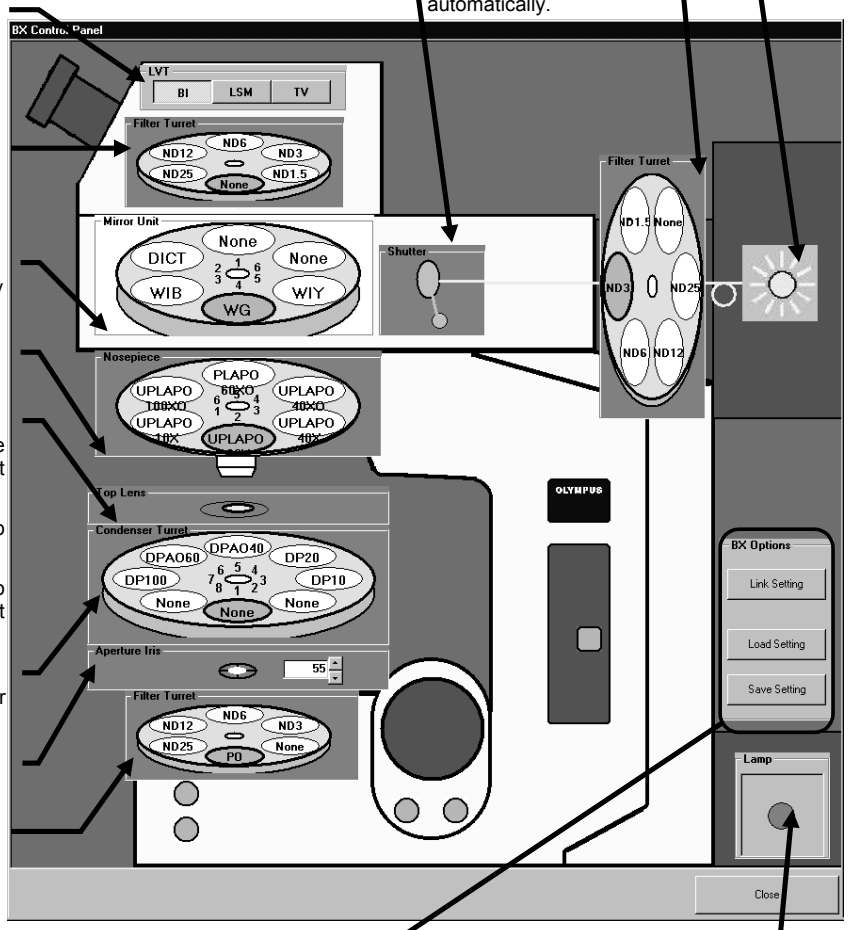


The top lens is disengaged from the light path.

[Condenser Turret] group box
Clicking the universal condenser automatically switches the turret.

[Aperture Iris] group box
Changes the AS value.

[Filter Turret] group box
Clicking the filter for transmitted light observation to be set switches the turret automatically.

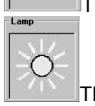


[BX Options] group box
Used for optional BX settings.
<Link Setting> button
Selects the function to change settings corresponding to the change of BX settings.
<Load Setting> button
Loads the BX settings already registered to reflect it.
<Save Setting>button
Saves the current BX settings.

[Lamp] group box
Clicking inside the box switches the TD lamp ON/OFF.



The TD lamp is set to OFF.



The TD lamp is set to ON.

- Clicking the <Link Setting> button displays the [Link Setting] dialog box as shown below.

Checking here links the objective in the [Nosepiece] group box with the condenser turret.

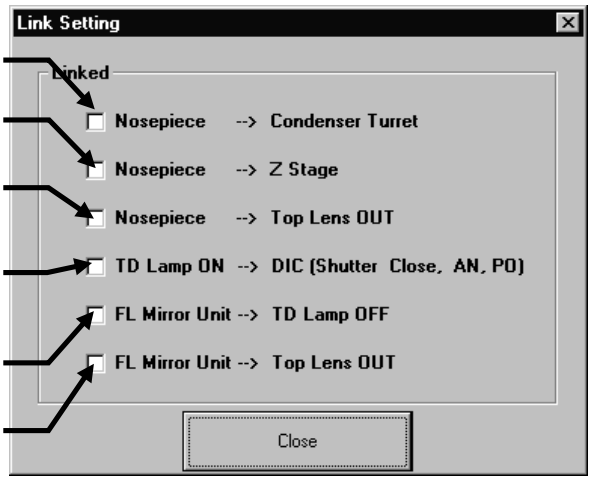
Checking here escapes the stage when the objective is selected and changed in the [Nosepiece] group box.

Checking here disengages the top lens from the light path when the objective of ×4 or lower magnification is selected in the [Nosepiece] group box.

Checking here closes the FL shutter and engages Analyzer and Polarizer into the light path when the TD lamp is set to ON in the [Lamp] group box.

Checking here sets the TD lamp to OFF when the fluorescent cube is selected in the [Mirror Unit].

Checking here disengages the top lens from the light path when the fluorescent cube is selected in the [Mirror Unit].



Click the desired check box to be checked.

Select the <Close> button to close the dialog box.

- Selecting the <Load Setting> button displays the [Open] dialog box as shown below.



When the setting file which you want to read is not displayed in the list box, use the [Look in:] drop-down list and select the drive or directory where the file is saved.

Select “BX Setting File (*.ini)” in the [Files of type:] drop-down list.

In the list box, select the setting file which you want to read.

Click the <OK> button to close the dialog box.

- Selecting the <Save Setting> button displays the [Save As] dialog box as shown below.



To change the save destination drive or directory, use the [Save in:] drop-down list.

Enter the setting file name into the [File name] text box.

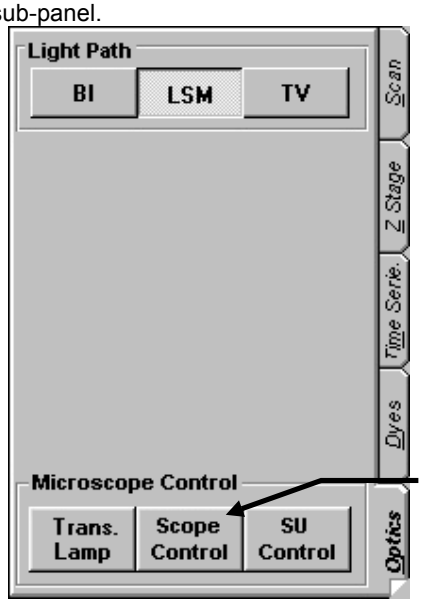
Select the <Save> button to close the dialog box.

1-3-2-3 Configuring the Microscope (AX70A)

When using the combination with the AX70A, the microscope and scan unit can be configured from the FLUOVIEW software.

Use the following procedure for this.

1. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.



<Scope Control> button
Click to start the system setup.

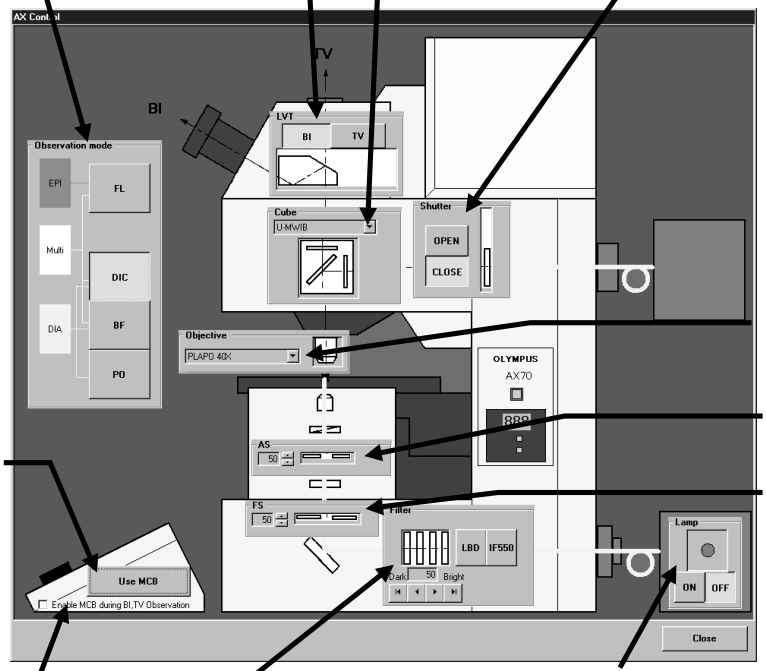
Fig. 1-20 [Optics] Sub-panel

2. Click the <Scope Control> button at the bottom of the panel. The window as shown below will appear.

[LVT] group box
Switches the light path.
Click <BI> button for visual observation or <TV> button for TV observation.
It is not necessary to change the setting when performing LSM observation.

[Cube] group box
Select the excitation filter from the drop-down list.
The selection from the drop-down list is possible when the <Reflected Fluorescence + Transmitted Differential Interference> or <Reflected Fluorescence> button is selected in the [Observation mode] group box.

[Observation mode] group box
Changes the microscopy mode.
Observation by combining more than one microscopy technique is also available.
The <FL> button performs reflected light observation.
The <DIC> button performs transmitted light DIC observation.
The <BF> button performs transmitted light brightfield observation.
The <PO> button performs polarized light observation.



[Shutter] group box
Opens or closes the shutter of the transmitted light lamp.
The shutter opens when <OPEN> button is selected.
The shutter closes when <CLOSE> button is selected.

[Objective] group box
Selects the objective from the drop-down list.

[AS] group box
Changes the value of AS.

[FS] group box
Changes the value of FS.

<Use MCB> button
Select to set the items other than those shown in the [AX Control] window by using the U-MCB.
After the setup using the U-MCB, click this button again to apply the set-up contents in the [AX Control] window.

[Enable MCB during BI, TV Observation] check box
The operation with MCB is possible when this check box is checked and the current observation mode is BI/TV observation.

[Filter] group box
<LBD> and <IF550> buttons
Selects whether color correction filter is engaged or not.
Click < |◀ > button to make the filter darker.
Click < ◀ > button to darken the filter by one step.
Click < ▶ > button to brighten the filter by one step.
Click < ▶| > button to make the filter brighter.

[Lamp] group box
Switches the transmitted lighting lamp ON or OFF.
The lamp is ON when <ON> button is selected.
The lamp is OFF when <OFF> button is selected.

3. After completing the setup, click the <Close> button.

TIP

To control the microscope with the U-MCB while keeping the software active, click the <Use MCB> button in the [AX Control] window before proceeding to the setup.
Selecting the <Use-MCB> again enables you to operate the microscope in this software.

TIP

During image acquisition, the <XY Repeat> and <Once> buttons are set as follows provided that the setting is not altered by the operator.

- [Observation mode] group box: The <DIC> button is selected.
- [Cube] group box: Set to OUT.
- [Shutter] group box: Set to “Close”
- [AS] group box: The value of AS is set to “100”. (This is variable depending on the objective type.)
- [FS] group box: The value of FS is set to “120”. (This is variable depending on the objective type.)
- [Lamp] group box: The transmitted light lamp is set to “OFF”.
- [FILTER] group box: The value of ND is set to “100”.
- [FILTER] group box: Both color correction filters are set to “OUT”.

1-3-2-4 Configuring the Filters

The barrier filters, excitation filter and beam splitter are set automatically to the light path according to the dyeing method selected for the specimen.

Use the following procedure to change these filters.

1. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.

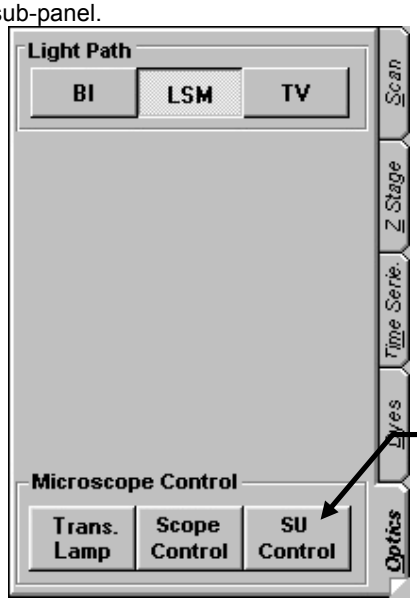


Fig. 1-21 [Optics] Sub-panel

[Laser Unit] group box
Shows the type of laser to be used.
(With the laser combiner operation, the laser type is set and displayed automatically.)
When the <On> button is pressed-in, the laser is oscillating the beam.
When the <Stby> button is pressed-in, the laser is not oscillating.
When the [Auto standby] check box is checked, the [After] text box appears below it.
When not using laser for a long time, in order to suppress useless electric-power consumption, we recommend you making <Stby> mode.
The laser oscillation stops when the time shown in this box has elapsed after the end of laser scanning.
The laser oscillation stops when the time shown in this box has elapsed after the end of laser scanning. The laser is suspended as standby mode (using Ar or Kr-laser) or the laser oscillation stops (using UV-Ar laser)
When the time shown in this box has elapsed after the end of laser scanning.

2. Click the <SU Control> button at the bottom of the panel. The window as shown below will appear.

[Excitation DM] and [Beam splitter] drop-down lists
Change the excitation filter and beam splitter of each channel.

[Confocal Aperture]
The optimum confocal aperture is set automatically to each channel according to the dye and objective in use, and the set aperture number is displayed.

Clicking this area causes the [Microscope Configuration] window to appear.

[Barrier Filter] drop-down list
Changes the barrier filter of each channel.

[TD Unit] group box
Shows the transmitted light detection.

[Dyes]
Shows the dyeing method set for each channel.

[Z Resolution]
Shows the Z resolution set for each channel.

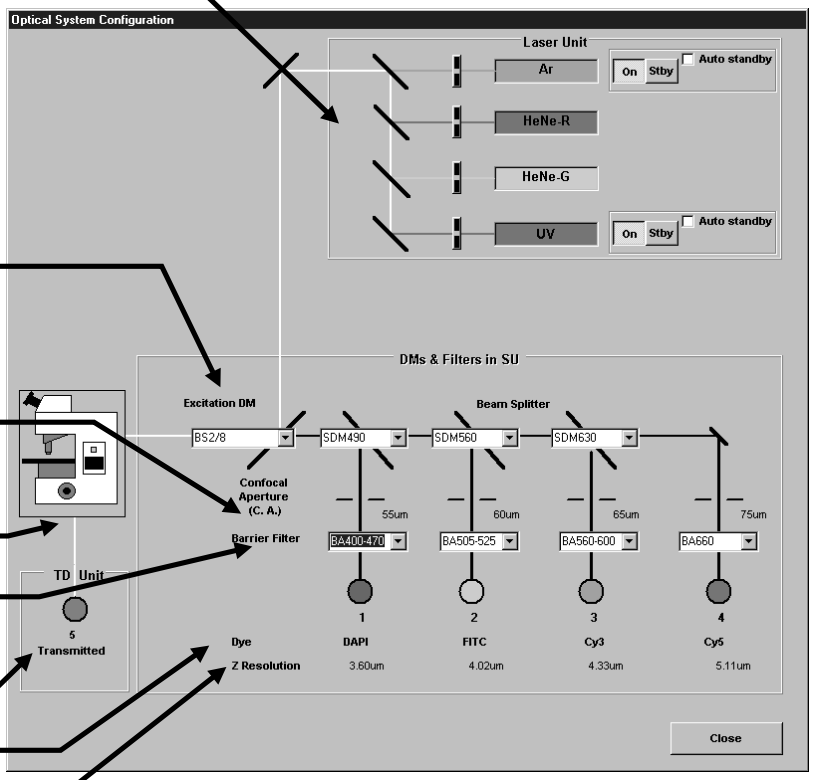


Fig. 1-22 [Optical System Configuration] Window

3. To change the displayed filter types, use the [Excitation DM], [Beam splitter] and/or [Barrier Filter] drop-down lists.

4. After completing the system setup, click the <Close> button to close the window.

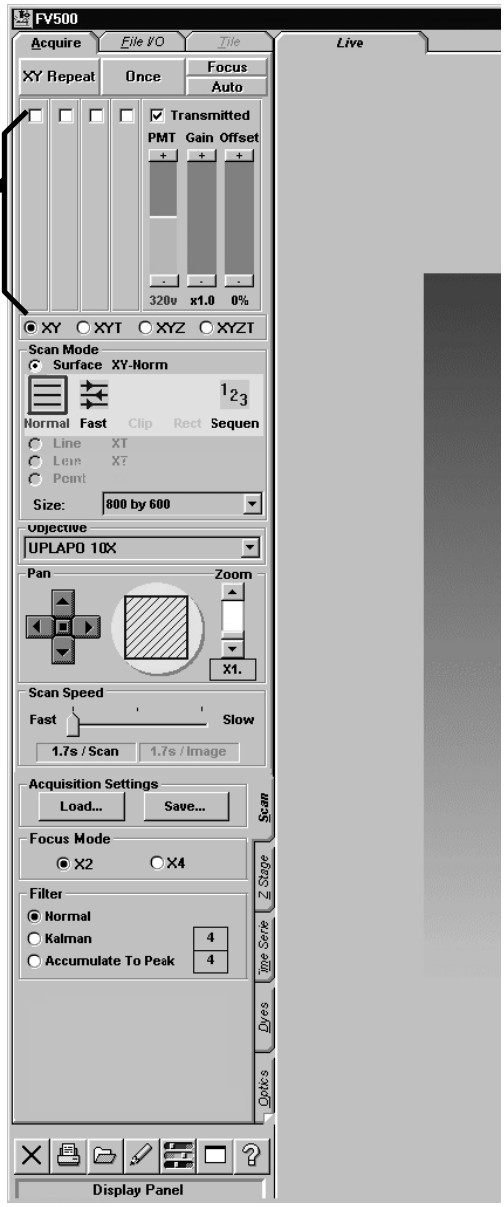
1-3-2-5 Setting the C.A. Diameters

The pinhole diameters are set automatically according to the selected dyeing method.

Use the following procedure to change the pinhole diameters.

1. Display the [Acquire] panel.

[Ch] group box
Sets whether the image of each channel is to be acquired or not, the PMT voltage, Gain and Offset.
Right-clicking the mouse inside this group box displays the [Ch] group boxes of all channels.

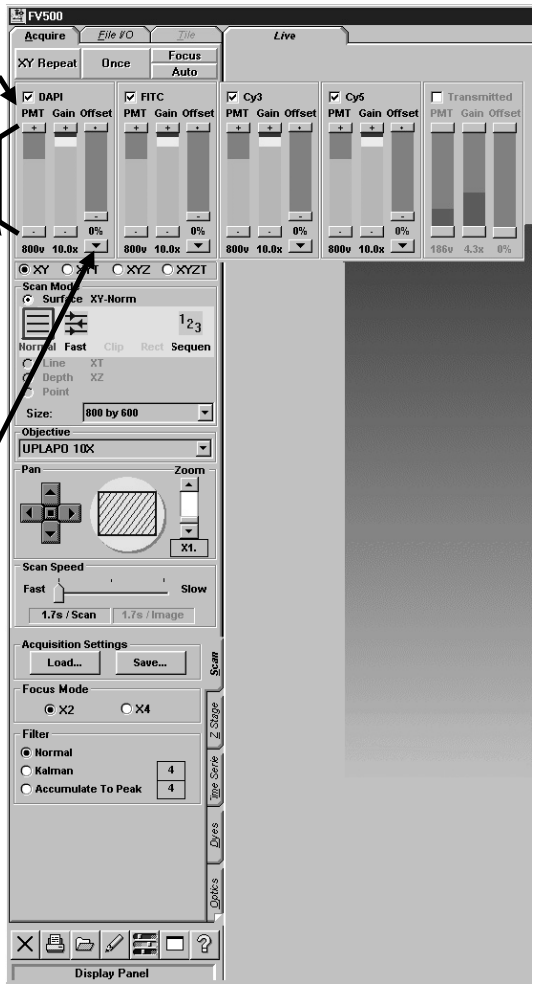


2. Right-click the mouse inside the [Ch] group box to display the [Ch] group boxes of all channels.

[Ch1], [Ch2], [Ch3], [Ch4] and [Ch5] group boxes
Select the channels from which you want to acquire images.

[PMT], [Gain] and [Offset] LED sliders
Set these values independently.
Increasing the PMT Voltage improves the sensitivity.
Increasing the Gain brightens the image.
Increasing the Offset darkens the image.

< ▼ > button
Click to display the pinhole diameter and the laser ND filter setting.



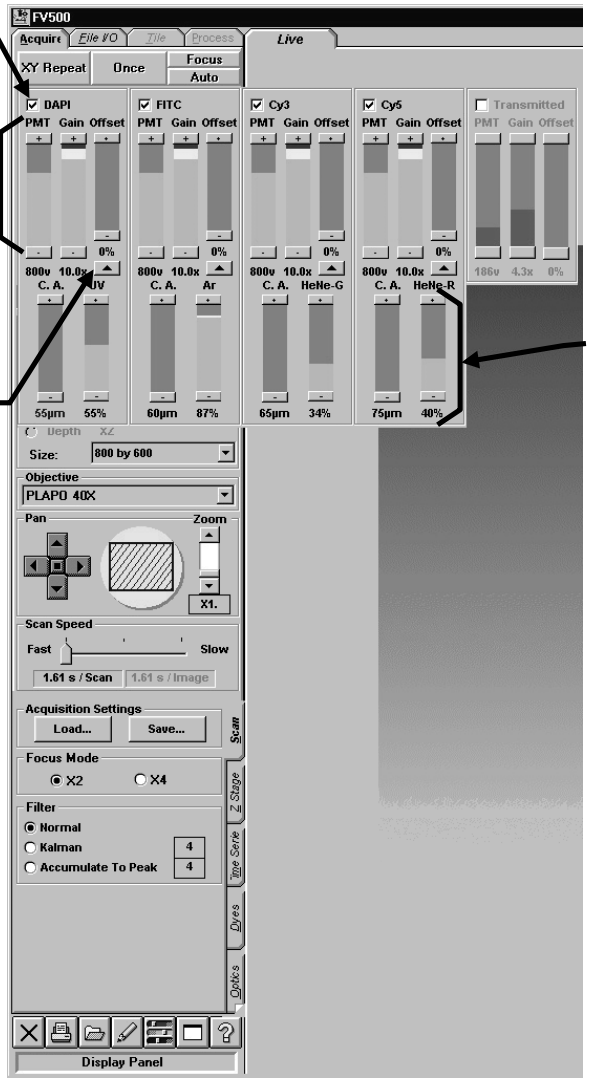
3. Click the < ▼ > button of the channel you want to change the pinhole diameter.

   The group boxes for setting the pinhole diameters and laser ND filter values of the channels are displayed below the [Ch] group box. (They are not displayed for channels set for transmitted light observation.)

[Ch1], [Ch2], [Ch3], [Ch4] and [Ch5] group boxes
Select the channels from which you want to acquire images.

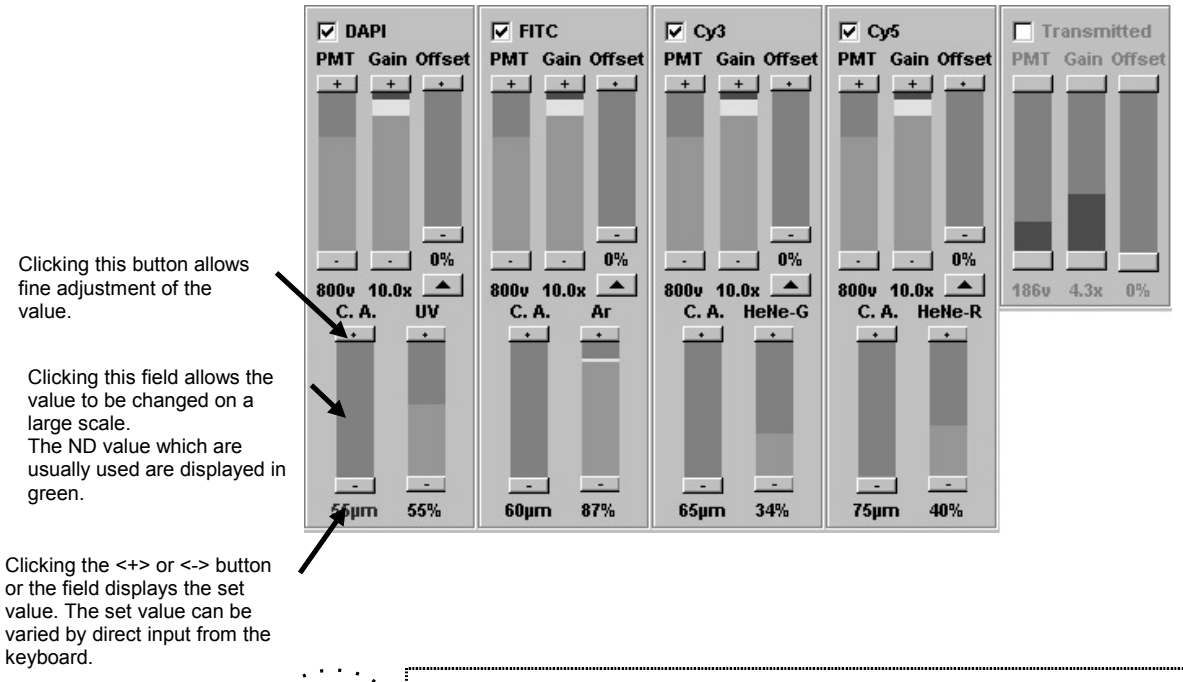
[PMT], [Gain] and [Offset] LED sliders
Set these values independently.
Increasing the PMT Voltage improves the sensitivity.
Increasing the Gain brightens the image.
Increasing the Offset darkens the image.

< ▲ > button
Click to close each group box showing the pinhole diameter and laser ND filter value. The pinhole diameter and laser ND filter value are valid even before the group box is closed by clicking the < ▲ > button.



[C.A.] and Laser ND LED sliders
Set these values independently.
The laser type used with each channel is displayed below its Laser ND LED slider.

4. While observing the images in the [Live] panel, change their pinhole diameter settings.



TIP

Each click of the <+> or <-> button of the [C.A.] LED slider increases or decreases the pinhole diameter by 5 nm.

Each click of the slider section of the [C.A.] LED slider varies the pinhole diameter by 25 nm.

Each click of the <+> or <-> button of the Laser ND LED slider increases or decreases the laser ND value by 1%.

Each click of the slider section of the Laser ND LED slider varies the laser ND value by 5%.

TIP

To set to the Confocal Aperture value to its primal value, click the <Apply> button in the [Dyes] sub-panel of the [Acquire] panel.

2 APPLIED OPERATIONS

2-1 General Operation Procedure

This section describes the general image acquisition procedure with the aim to get accustomed with the operation.

Begin using the FLUOVIEW system by acquiring an image or opening an image from a file. The procedures for the subsequent operations such as image processing are not in question here. For the detailed operation method of each item in the procedure, see the section specified in parentheses (( )).

NOTE

The following is the general operation procedure of FLUOVIEW. Many other functions that are not shown in the following are also available. Please also study their description.

Turn power ON and start the FLUOVIEW software.
(Sections 1-2-1 & 1-2-2)

A

Acquire an image.
(According to the selected observation mode)

For detailed operation procedures for image acquisition, see section 2-1-1, “Image Acquisition Procedure (Section A)”.

Open an image in a file.
(Section 2-3-2)

Process the image.

Filtering (Section 2-5-1)
Contrast conversion (Section 2-5-2)
Inter-image operation (Section 2-5-3)

Observe the image.

- **Observation of image shape**
  Image display in simulated colors (Section 2-4-1)
  LUT change (Section 2-4-2)
  Simultaneous display of multi-channel images (Section 2-4-4)
  Side-by-side image display (Section 2-4-7)
  Magnified/reduced image display (Section 2-4-9)
  Stereo 3D image display (Section 2-8-3)
  Color-eyeglass 3D image display (Section 2-8-4)
  Animation display (Section 2-8-2)
  Continuous display (Frame-by-frame display) (Section 2-8-1)
- **Observation of image change over time**
  Side-by-side image display (Section 2-9-1)
  Continuous display (Frame-by-frame display) (Section 2-9-2)

Measure the image.

- **Measurement of image shape**
  Length measurement(Section 2-6-3-1)
  Area measurement (Section 2-6-3-2)
- **Measurement of image intensity value and distribution**
  Intensity on a line (Line profile) (Section 2-6-1-1)
  Intensity on a plane (Bird’s eye view) (Section 2-6-1-2)
  Intensity distribution (Section 2-6-2)

Save the image.
(Section 2-3-1)

Compile the presentation data.

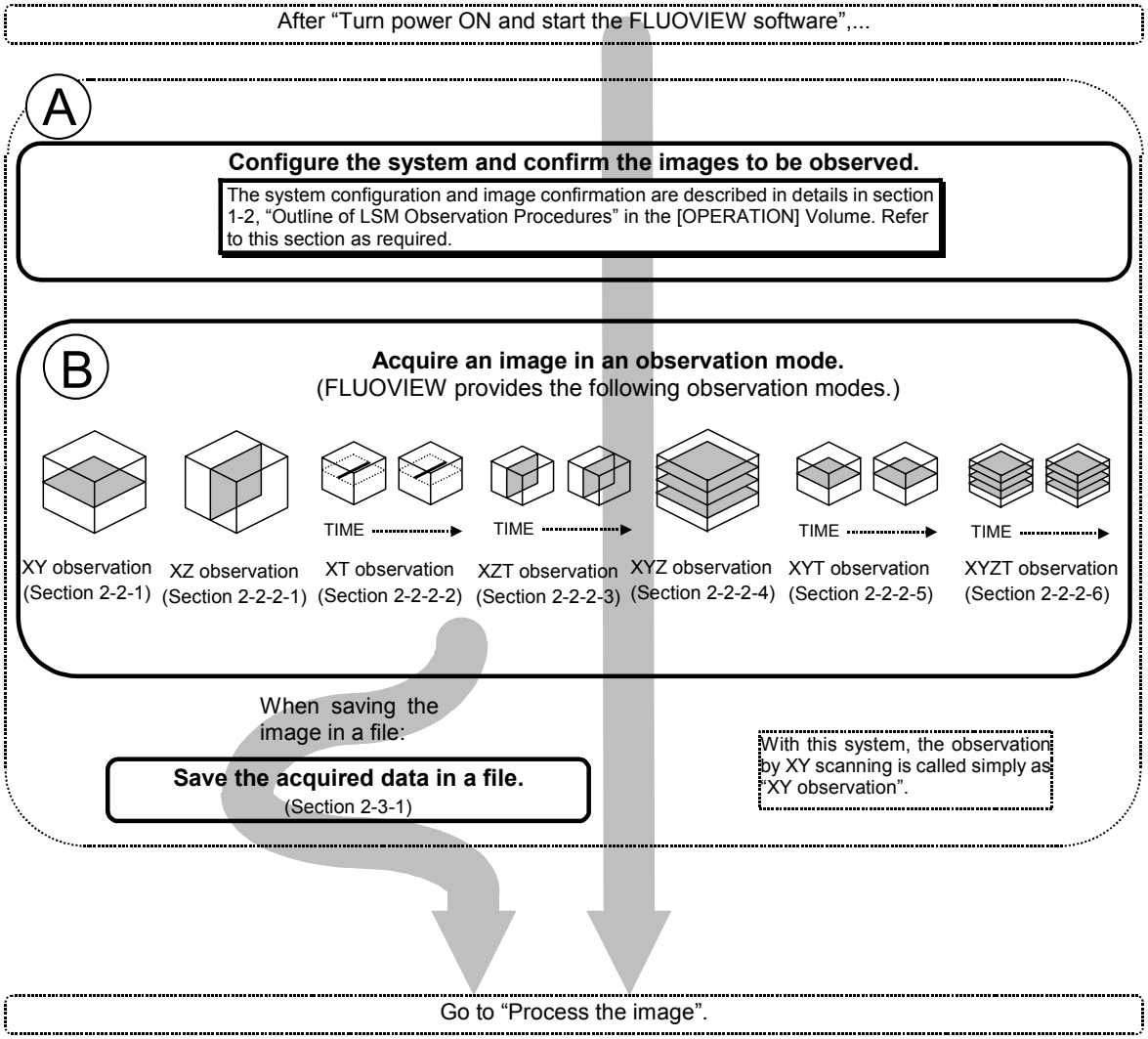
Drawing characters on image (Section 2-11-1)
Drawing pictures on image (Section 2-11-2)
Drawing scales on image (Section 2-11-3)

Output the image at the printer.
(Section 2-12)

Exit from the FLUOVIEW software and turn power OFF.
(Sections 1-2-11 & 1-2-12)

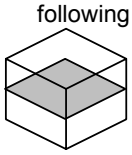
2-1-1 Image Acquisition Procedure (Section (A))

This section describes the procedure for acquiring images. See sections 2-2 and after for the actual operation methods. The detailed operation methods of each item in the procedure are described in the section specified in parentheses (( )).



2-1-2 Image Acquisition Procedure in an Observation Mode (Section (B))

As an example of “Acquire an image in an observation mode”, this section describes the procedure in the XY observation mode. For the procedures in other observation modes, see section 2-2-2, “Image Acquisition in Other Observation Modes” as well as the following procedure.



XY observation
(Section 2-2-1)

After “Configure the system and confirm the images to be observed”...

B

Set the acquisition parameters.
(Section 2-2-1-4)

- Observation mode
- Scanning speed
- Image brightness
- Multiple-section to be observed
- Area to be observed

Acquire an image in the XY observation mode.
(Section 2-2-1-5)

When noise is noticeable:

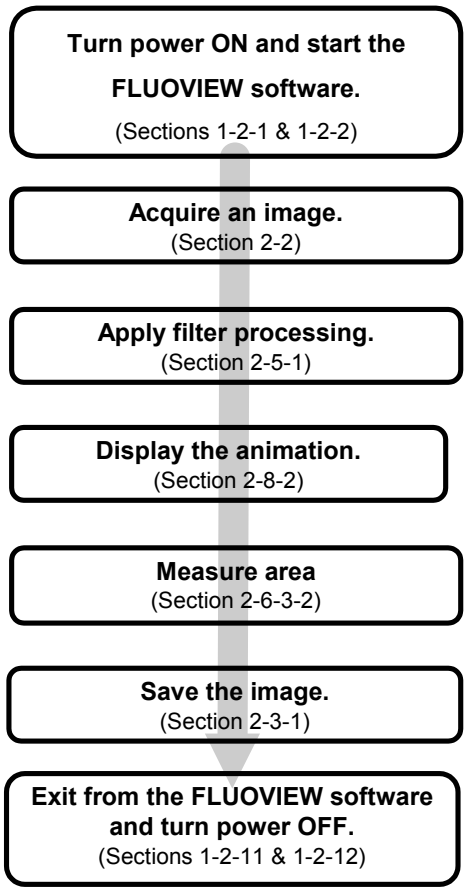
Perform accumulation.
(Section 2-2-1-6)

Go to “Save the acquired data in a file.” or “Process the image”.

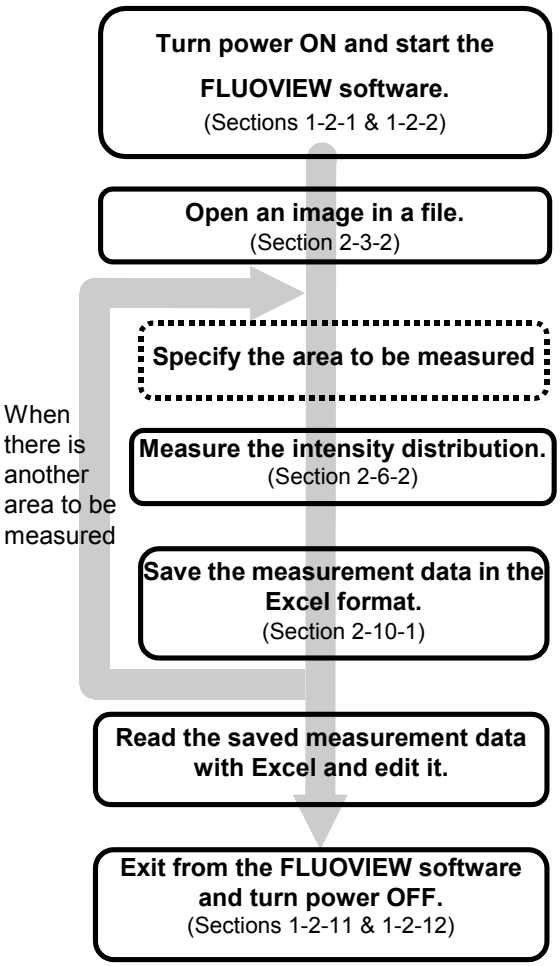
2-1-3 Examples of Operation Procedures

Begin using the FLUOVIEW system by acquiring an image or opening an image in a file. The procedures for the subsequent operations such as image processing are not in question here. For the detailed operation method of each item in the procedure, see the section specified in parentheses (( )).

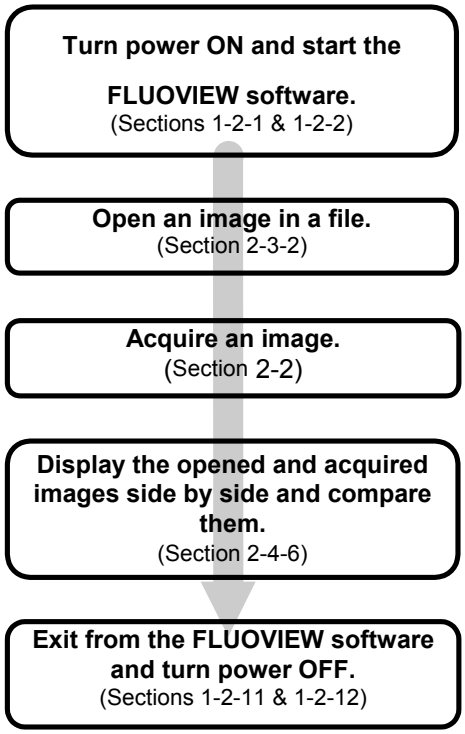
Example 1) To perform XYZ observation of a cell, apply filter processing to the image, display animation to identify the cell shape, then calculate the area, and save the obtained image data:



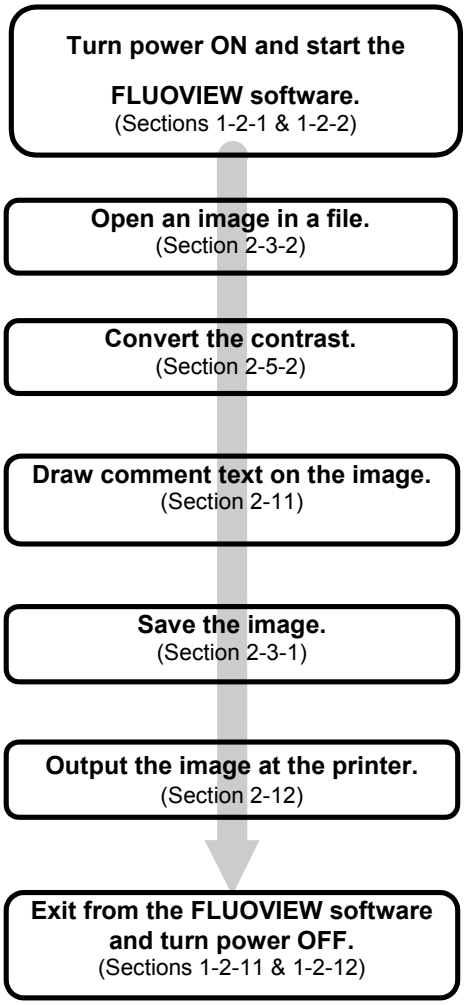
Example 2) To open a previously acquired image in a file, measure the intensity distribution and edit data with Excel:



Example 3) To acquire an image and compared it with a previously acquired image:



Example 4) To open an image in a file, improve its contrast and create a presentation image by entering comment, etc.



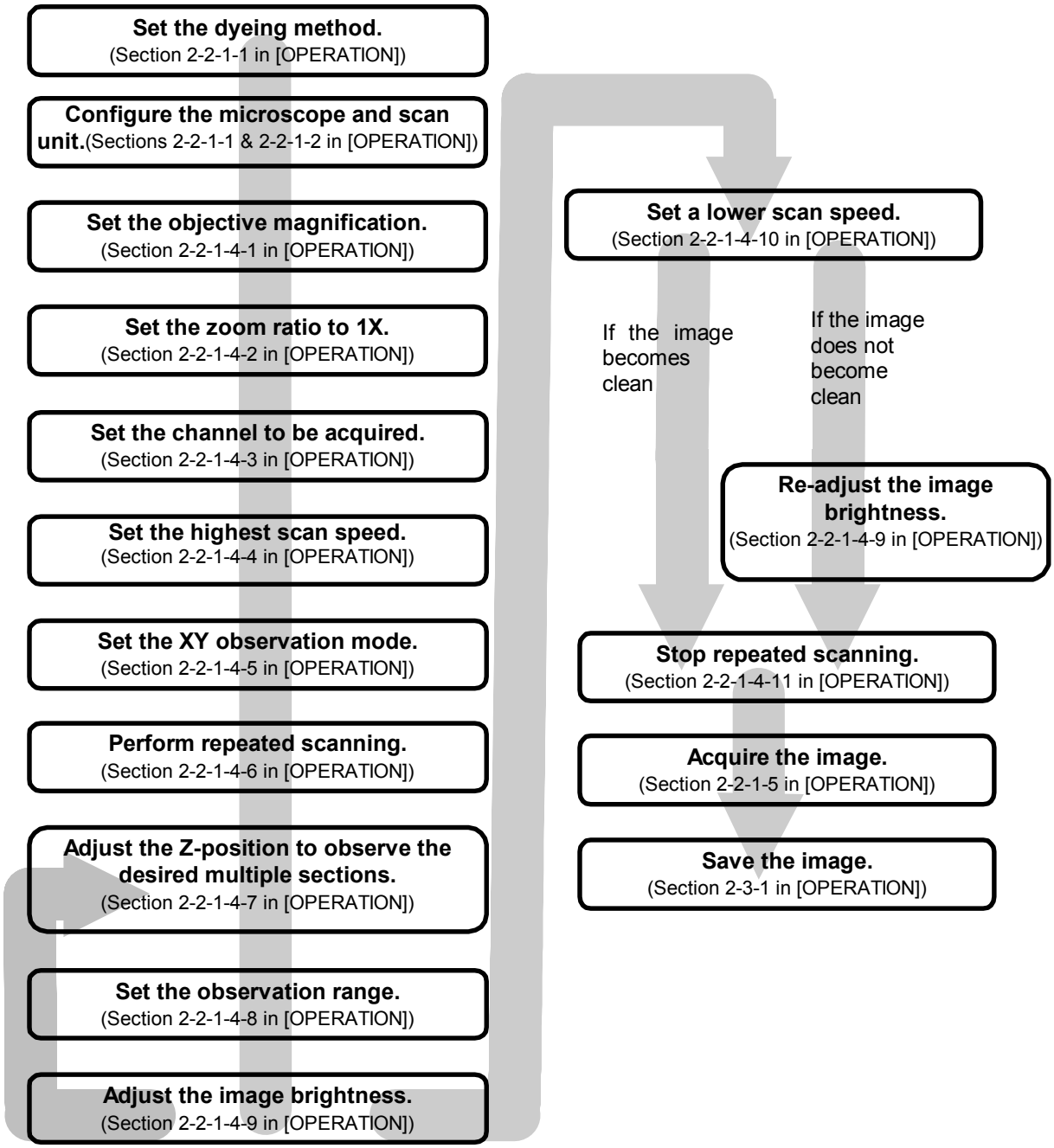
2-2 Image Acquisition

Confirm the image to be acquired using the microscope, and acquire its image using FLUOVIEW. The image can be saved in a file as required.

NOTE **Do not move FLOUVIEW FV500 Menu while acquiring an image.**

2-2-1 Image Acquisition in XY Observation Mode

This section describes the basic operation procedure from the system configuration to the image acquisition in the XY observation mode and image saving in a file as shown in the following chart. The details of each operation will be described in the subsequent sections.



2-2-1-1 Configuring the Microscope

Set the light path so that the image can be observed through the microscope.

1. Display the [Acquire] panel.

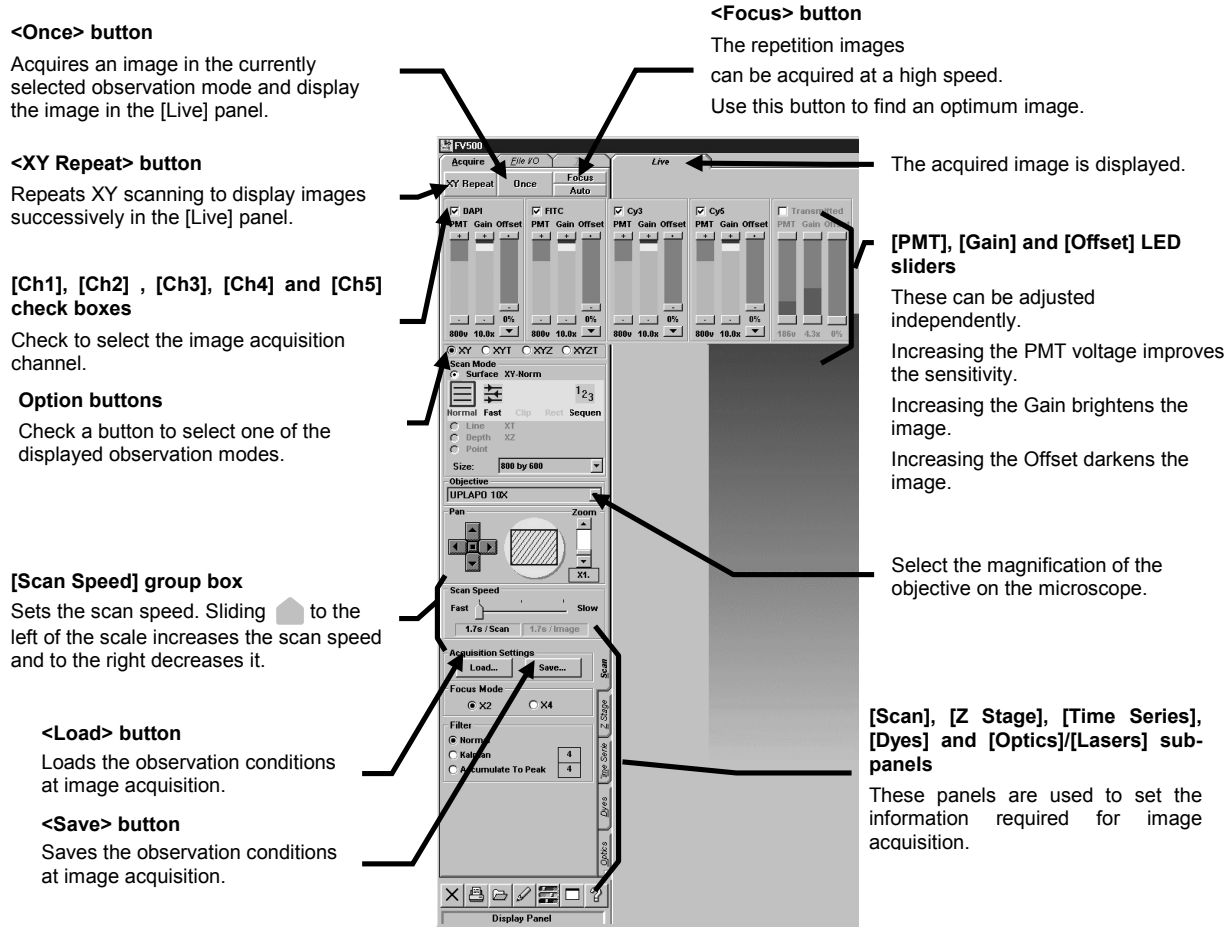


Fig. 2-1 [Acquire] Panel

2. Setting the dyeing method

1) From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

[Assign dyes manually] check box

Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

[Available Dyes] list box

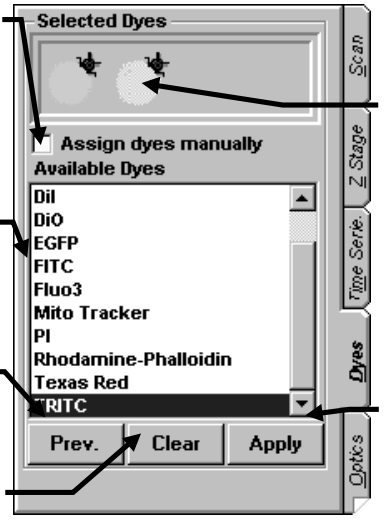
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Prev.> button

Sets the dyeing method which was set last time by clicking the <Apply> button.

<Clear> button

Clear the set dyeing method.



Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

<Apply> button

Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.

Fig. 2-2 [Dyes] Sub-panel

2) Select the specimen dyeing method by dragging desired dye names in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

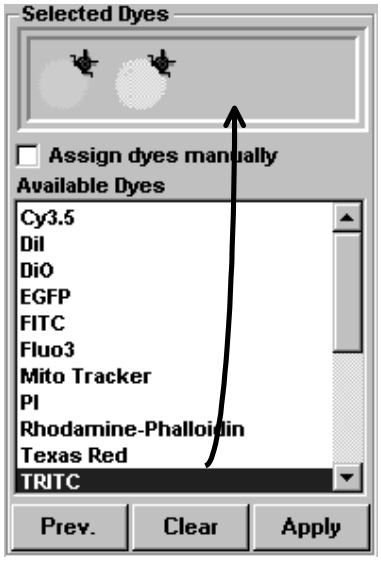


Fig. 2-3 [Dyes] Sub-panel

3) Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

TIP

When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the changed filter. And the dyeing method is shown in the [Ch] group box.
The Confocal Aperture value is also set automatically according to the wavelength and the objective per channels.

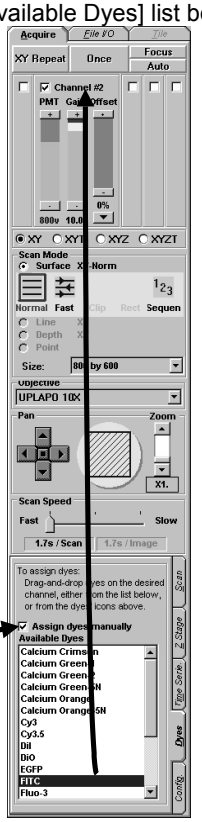
TIP

If you change the objective, click the <Apply> button in the [Dyes] sub panel. The Conforcal Aperture value is set appropriately.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

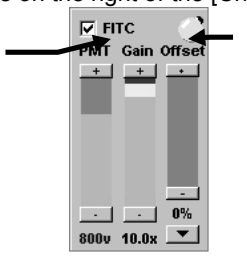
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set



Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

3. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.

<Scope Control> button
Displays useful information for the system setup.

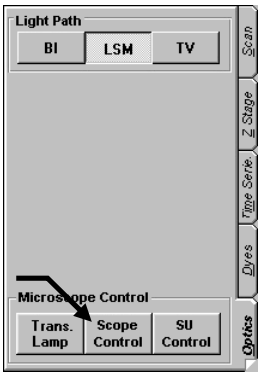


Fig. 2-4 [Optics] Sub-panel

4. Select the <Scope Control> button at the bottom of the panel. The window as shown below will appear (in case of a combination with the IX).

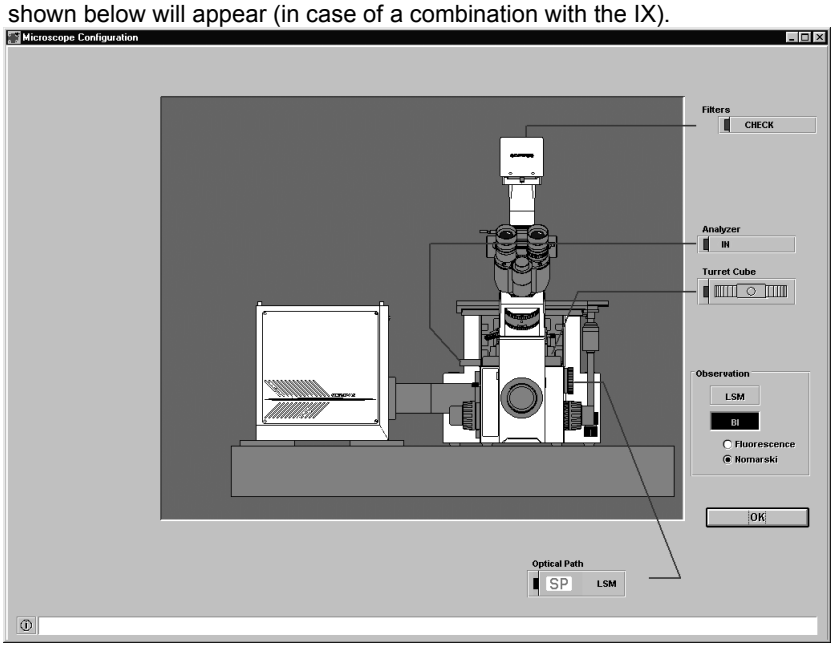
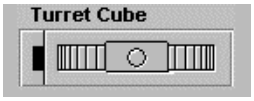


Fig. 2-5 [Microscope Configuration] Window



<BI> button

5. Select the <BI> button in the [Observation] group box, and select the microscopy from the option buttons below it. The points where system setting is to be changed for microscope observation will blink in red.



6. Change the system configuration (setup of cube turret, etc.) by following the guidance given by the red blinking light.

TIP For the operation of the lever, see section 1-1-1, “Microscope” in this volume.

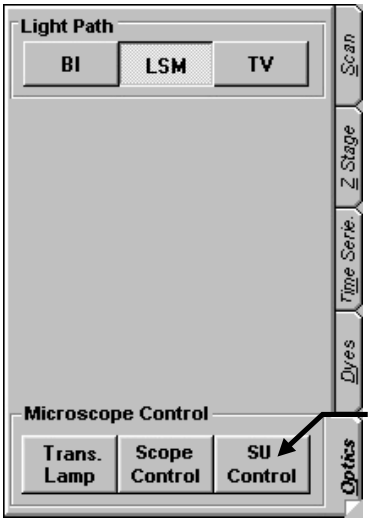
NOTE **The [Microscope Configuration] window is designed to give guidance on system configuration. The red blinking light does not stop even after the indicated configuration point has been changed.**

7. While looking into the microscope, move the stage and check the observed image.

2-2-1-2 Setting the Filters

The excitation filter, spectral filter and barrier filters are set automatically to the light path according to the dyeing method selected for the specimen.
To change filters, use the following procedure.

1. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.



<SU Control> button
Click to display the filter and laser types to be set.
(Combination with the laser combiner sets up the laser automatically.)

Fig. 2-6 [Optics] Sub-panel

2. Click the <SU Control> button at the bottom of the panel. The window as shown below will appear.

[Laser Unit] group box

Shows the type of laser to be used.
(With the laser combiner operation, the laser type is set and displayed automatically.)
When the <On> button is pressed-in, the laser is oscillating the beam.
When the <Stby> button is pressed-in, the laser is not oscillating.
When the [Auto standby] check box is checked, the [After] text box appears below it.

When not using laser for a long time, in order to suppress useless electric-power consumption, we recommend you making <Stby> mode.
The laser oscillation stops when the time shown in this box has elapsed after the end of laser scanning.

The laser oscillation stops when the time shown in this box has elapsed after the end of laser scanning. The laser is suspended as standby mode (using Ar or Kr-laser) or the laser oscillation stops(using UV-Ar laser)

When the time shown in this box has elapsed after the end of laser scanning.

[Excitation DM] and [Beam splitter] drop-down lists

Change the excitation filter and beam splitter of each channel.

[Barrier Filter] drop-down list

Changes the barrier filter of each channel.

[TD Unit] group box

Shows the transmitted light detection.

[Dyes]

Shows the dyeing method set for each channel.

[Z Resolution]

Shows the Z resolution set for each channel.

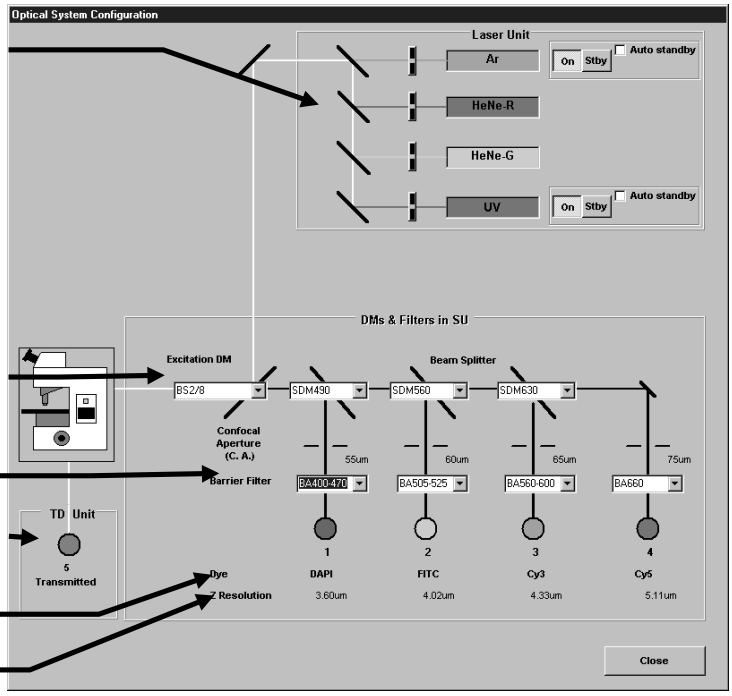


Fig. 2-7 [Optical System Configuration] Window

3. To change the displayed filter types, use the [Excitation DM], [Beam splitter] and/or [Barrier Filter] drop-down lists.

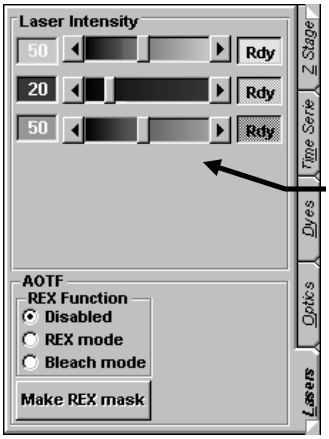
4. After completing the setup, click the <Close> button.

2-2-1-3 Setting the ND Filters

- When the laser combiner is used

1. Display the [Acquire] panel.

   In the [Lasers] group box of the [Lasers] sub-panel, set the ND filter scale of each laser according to the levels of specimen brightness, fluorescence crosstalk and fading.



[Laser Intensity] group box
Set the ND of the lasers in the scales.
The number of displayed lasers varies depending on the number of input channels.

When the HeNe Green laser is used, try ND of 50% by setting the [Intensity] scale in the [Lasers] group box to 50.

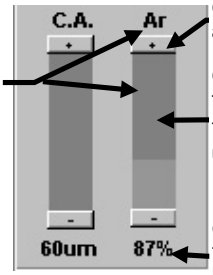
With other lasers, try ND of 10% first.

One Point!

After the dyeing method has been set with the [Dyes] sub-panel, the ND filters can be set using the [Ch] group box in the upper part of the [Acquire] panel.

1. In the upper part of the [Acquire] sub-panel, open the [Ch] group box for the ND to be changed.
2. Click the <More> button.
   The field as shown below is displayed below the [Ch] group box.

Display of the optimum laser and ND value which are set automatically according to the selected dyeing method.



Clicking this button allows fine adjustment of the ND value.

Clicking this field allows the ND value to be changed on a large scale.

The ND values which are usually used are displayed in green.

Display of the ND value set by clicking the <+> and <-> buttons or the field. The set ND value can also be changed by entering its value directly from the keyboard.

3. Vary the ND value using the Laser LED slider.
   Each click of the laser ND <+> or <-> varies the laser ND value by 1%.
   Each click of the laser ND slider varies the laser ND value by 5%.

2-2-1-4 Setting the Observation Condition

1 Setting the Objective Magnification

1. From the drop-down list on the center of the [Acquire] panel, select the objective being used with the microscope.



NOTE **With a combination using a microscope other than the AX70A, the measurement results will be inaccurate if the objective magnification set here does not match the actual magnification of the objective in use.**

TIP If you change the objective, click the <Apply> button in the [Dyes] sub panel. The Conforcal Aperture value is set appropriately.

2 Setting the Zoom Ratio to 1X

1. Use the [Zoom] scale in the [Acquire] panel to set the zoom ratio to “X1”.

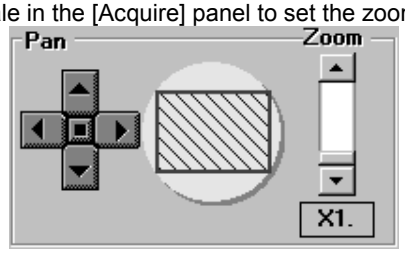
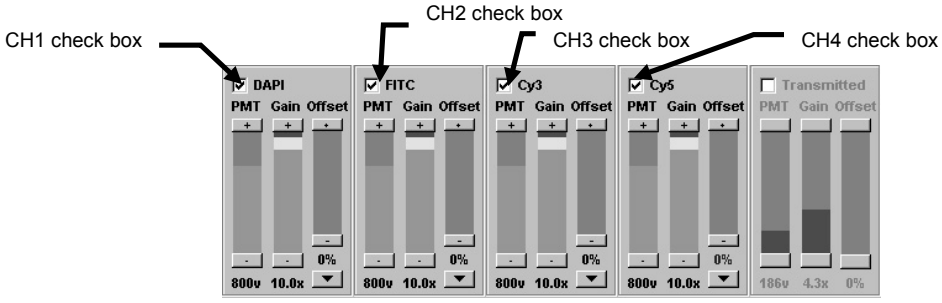


Fig. 2-8 [Pan]/[Zoom] Group Box

Using the UV-Ar laser, set the zoom ratio to “X2”.

3 Setting the Channels

1. In the Channel 1 group box, check the check box showing the applicable dyeing method to make the image acquisition ready.
2. In the Channel 2 group box, check the check box showing the applicable dyeing method to ready the image acquisition.
3. In the Channel 3 group box, check the check box showing the applicable dyeing method to ready the image acquisition.
4. In the Channel 4 group box, check the check box showing the applicable dyeing method to ready the image acquisition.



TIP

To display the information on all channels simultaneously, right-click the boundary between channel display boxes.
Click the boundary again to return to the original display.

4 Setting the Highest Scan Speed

Set the scan speed to the fastest speed by using the scale in the [Scan Speed] group box in the [Acquire] panel

[Scan Speed] group box

Set the scan speed by clicking a point on the scale line.



TIP

The focus mode makes it possible to increase the line skipped scan speed.

From the page tabs on the bottom right of the [Acquire] panel, select the [Scan] sub-panel.

Select either option button in the [Focus Mode] group box.

[Focus Mode] group box

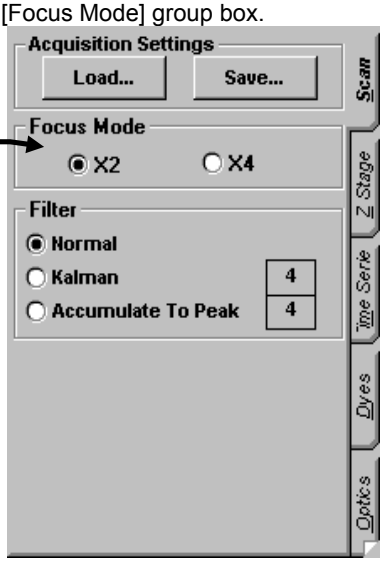
[X2] option button

Acquires image at twice the highest speed.

[X4] option button

Acquires image at 4 times the highest speed.

Increasing the number of divided images in the [Display] panel, line skipped scan at 4 times (Focus) cannot be done.



The focus mode is enabled when acquiring images using the <Focus> button.

NOTE

The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.

5 Setting the XY Observation Mode

1. In the [Scan Mode] group box in the [Acquire] panel, select the [Surface] option button.
2. In the [Acquire] panel, select the XY observation mode option button.

6 Repeated Scanning Operation



<XY Repeat> button

1. Select the <XY Repeat> button. The acquired image will be displayed in the [Live] panel.



<Focus> button

TIP

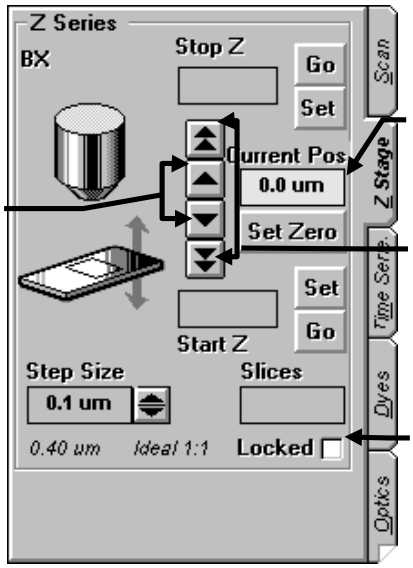
Use the <FOCUS> button to acquire image at an even higher speed. If the specimen is already being scanned, stop scanning with the <STOP SCAN> button before selecting the <XY Repeat> button.
The Focus function reduces the scanning time by line skipped scan. As a result, the acquired images become coarse.

7 Setting the Multiple sections to be Observed

While acquiring image, move the Z stage to select the multiple sections to be observed.

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

<Z stage fine adjustment> buttons
Displace the Z stage by 0.1 μm per step.



[Current Pos] text box
Shows the current position of the stage. The value can also be entered directly from the keyboard.

<Z stage coarse adjustment> buttons
Displace the Z stage by 1.0 μm per step.

[Locked] check box
Enables the Z motor operation.

Fig. 2-9 [Z Stage] Sub-panel

TIP

The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed.
See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.

Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

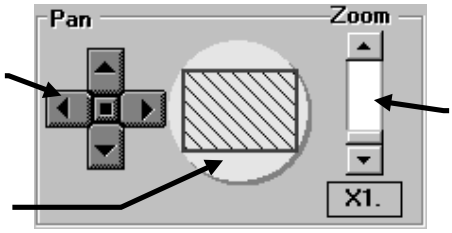
2. While observing the image in the [Live] panel, locate the plane to be observed by displacing the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

8 Setting the Area to be Observed

When the observation targets are concentrated in a narrow area or when observation of a specific area detail is required, the image of a limited area can be selected.

The 4 buttons represent directions, and clicking a button moves the acquired image area in the direction indicated by the button.
Clicking the square button on the center returns the acquired image area to the center.

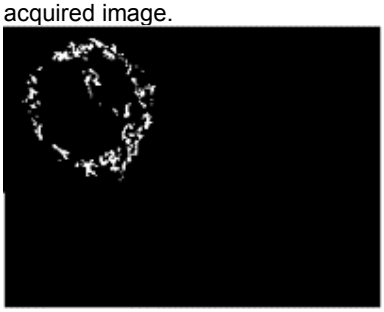
Click a point inside the circle to change the position of the acquired image area directly.



Clicking a point in the scale area to change the value on a large scale.
Clicking the top or bottom arrow button allows fine adjustment of the value.
Dragging the square knob allows the value to be changed directly.

Fig. 2-10 [Pan]/[Zoom] Group Box

For instance, let us assume that the observation target is deviated at the top left of the acquired image.



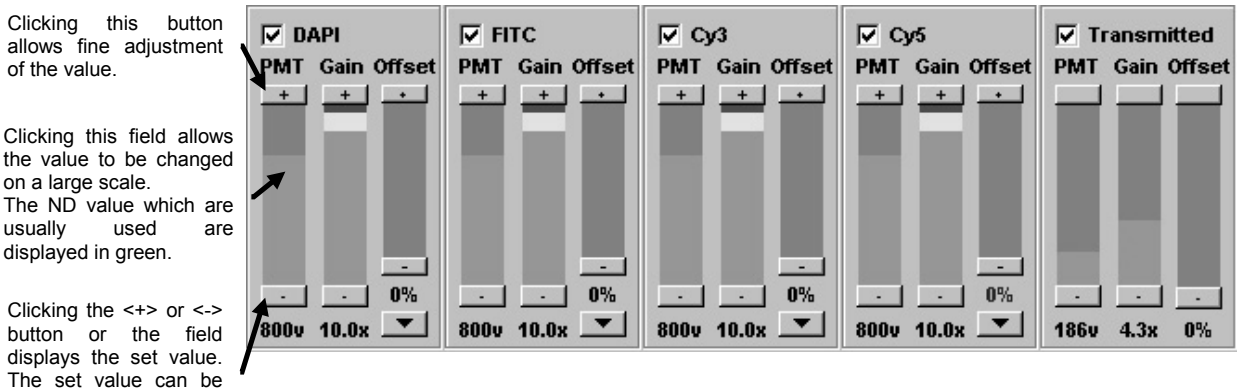


With this example, the area containing the observation target can be observed using the following procedure.

1. Increase the zoom ratio using the [Zoom] scale in the [Acquire] panel.

2. The light-blue circle to the left of the [Zoom] scale represents the field visible through the microscope, and the blue frame indicates the acquired image area. Move the blue frame inside the circle using the <Pan> buttons so that the desired observation targets are displayed in the [Live] panel.

9 Adjusting the image brightness



1. While observing the image in the [Live] panel, change the setting values of the **PMT voltage**[PMT], **Offset**[Offset] and **Gain**[Gain] in the [Acquire] panel. The functions of these parameters are as described below.

PMT Voltage	Increasing this value improves the sensitivity. However, too high a sensitivity makes image noise noticeable. If sufficient brightness cannot be obtained by setting the PMT voltage to 800 V, leave it as it is and increase Gain. This will usually provide a better result than using a PMT voltage over 800 V.
Offset	Darkens the image at the ratio set during image acquisition. (This value can be set independently from the Gain.)
Gain	Brightens the image at the ratio set during image acquisition. (This value can be set independently from the Offset.)

For example...

When the acquired image is dark or the observation targets are hardly visible, increase **PMT voltage** to improve the sensitivity. If the image resulting from this becomes too bright, first try decreasing **PMT voltage** slightly. If the image background is still too bright, increase **Offset** to darken the background. As this also reduces the brightness of the observation target, increase **Gain** as required so that the observation target is clearly visible.

Gamma Correction provides you more brightened image even if it was acquired with darkness.

See “2-4-2-2 LUT Graph Editing by Gamma Correction “ for details in this operation.

TIP

Each click of the <+> or <-> button of the [PMT] LED slider increases or decreases the PMT voltage by 5 V.

Each click of the slider section of the [PMT] LED slider varies the PMT voltage by 25 V.

In the transmitted channel case, each click of the <+> or <-> button of the [PMT] LED slider increases or decreases the PMT voltage by 1V.

In the transmitted channel case, each click of the slider section of the [PMT] LED slider varies the PMT voltage by 10V.

Each click of the <+> or <-> button of the [Gain] LED slider increases or decreases the Gain by X0.5.

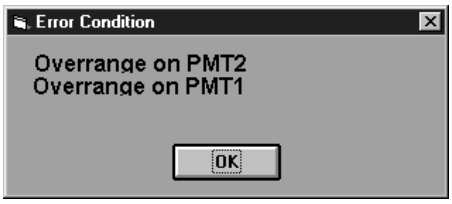
Each click of the slider section of the [Gain] LED slider varies the Gain by .X0.5.

Each click of the <+> or <-> button of the [Offset] LED slider increases or decreases Offset by 1%.

Each click of the slider section of the [Offset] LED slider varies the Offset by 5%.

NOTE

When the light incident to photomultiplier tube is too bright or PMT voltage is set to a high voltage, PMT Over warning may be displayed as shown below.



This warning is displayed to protect photomultiplier tube when the light incident to it exceeds a certain level. When it is displayed, decrease PMT voltage setting.

10 Setting a Lower Scan Speed

1. The scan speed can be decreased using the scale in the [Scan Speed] group box on the [Acquire] panel.

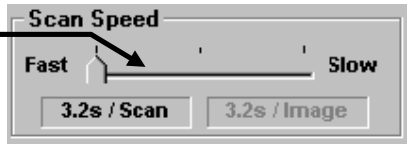
TIP

In general, setting a lower scan speed allows the acquired image quality to be improved.
However, a low scan speed also lengthens the time required for image acquisition.

NOTE

When the scan speed is decreased during fluorescence observation, the saturation of fluorescence may darken the image of certain types of specimens. In this case, increase the scan speed and increase the PMT Voltage or use accumulation in scanning.

[Scan Speed] group box
Set the scan speed by clicking a point on the scale line.



11 Stopping Repeated Scanning

1. After the brightness and gain have been adjusted, select the <STOP SCAN> button in the [Acquire] panel to stop scanning temporarily.



2-2-1-5 Acquiring Image



<Once> button

1. Select the <Once> button. The acquired image will be displayed in the [Live] panel.

2-2-1-6 Acquiring Image in Accumulation Mode

When the image is dark or noisy, use an accumulation mode in image acquisition to improve the image quality.

Kalman Accumulation and Peak Accumulation

- The Kalman accumulation acquires images for the specified number of times while averaging the images. This operation is effective for reduction of noise.
- The Peak accumulation acquires images for the specified number of times while adding the images, and stops image acquisition when any intensity value on the image reaches the peak (4095). This operation is effective for acquiring an image with the dark lower part in the XYZ observation and observation of an extremely dark image.

Kalman Accumulation Algorithm

Every time an image is acquired, the pixel values are rewritten based on the following formulae, where it is assumed that;

n: number of image acquisitions;
I(n): Result of n-times of Kalman accumulations (Intensity values of pixels);
I(new): New intensity value obtained after every image acquisition.

The result of the first Kalman accumulation is identical to the result of ordinary image acquisition:
I(1) = I(new)
The result of the n-th (n > 1) Kalman accumulation is:
I(n) = (I(n-1) * (n+1) + I(new)) / n

1 Acquiring Image in Accumulation Mode (Frame mode)

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Scan] sub-panel.

[Filter] group box
Select the accumulation mode.
Two accumulation modes, [Kalman] and [Accumulate To Peak] are available.

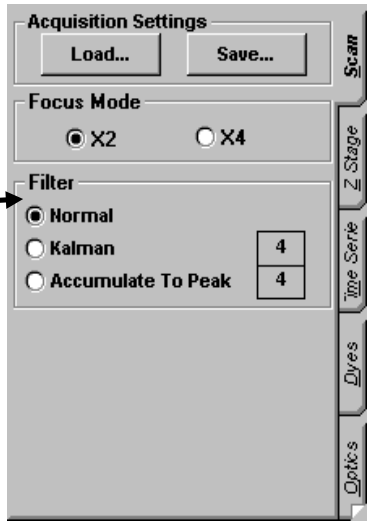
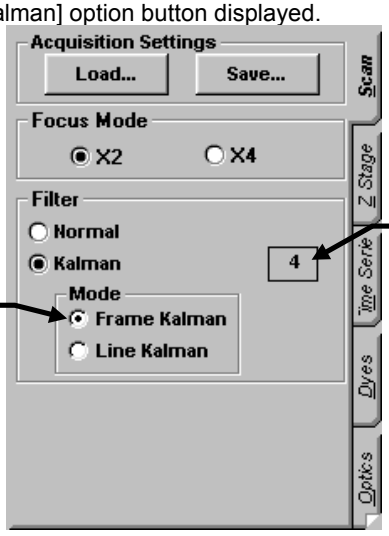


Fig. 2-11 [Scan] Sub-panel

2. In the [Filter] group box, select the [Kalman] or [Accumulate To Peak] option button.
3. When [Kalman] is selected, enter the accumulation count in the text box. And select the [Frame Kalman] option button displayed.



Enter the accumulation count.

[Frame Kalman] option button

TIP

The accumulation count can be set up a maximum of 63 times.
When 0 is set as the number of times of accumulation, an ordinary image acquisition is to be performed.

4. When [Accumulate To Peak] is selected, enter the addition count in the text box.

5. Click the <Once> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.

2 Acquiring Image in Accumulation Mode (Line mode)

NOTE **Image acquisition in the line mode can be performed when you use the FV300 system with AOTF (FV5-COMBA).**

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Scan] sub-panel.

[Filter] group box
Select the accumulation mode.
Two accumulation modes, [Kalman] and [Accumulate To Peak] are available.

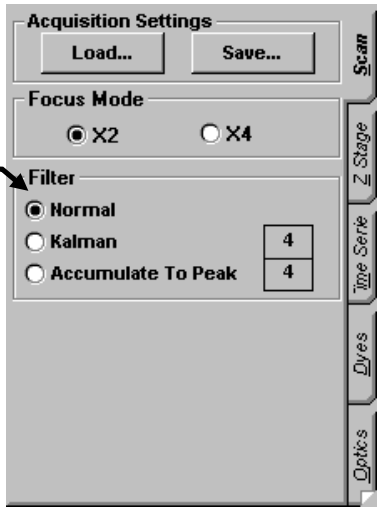
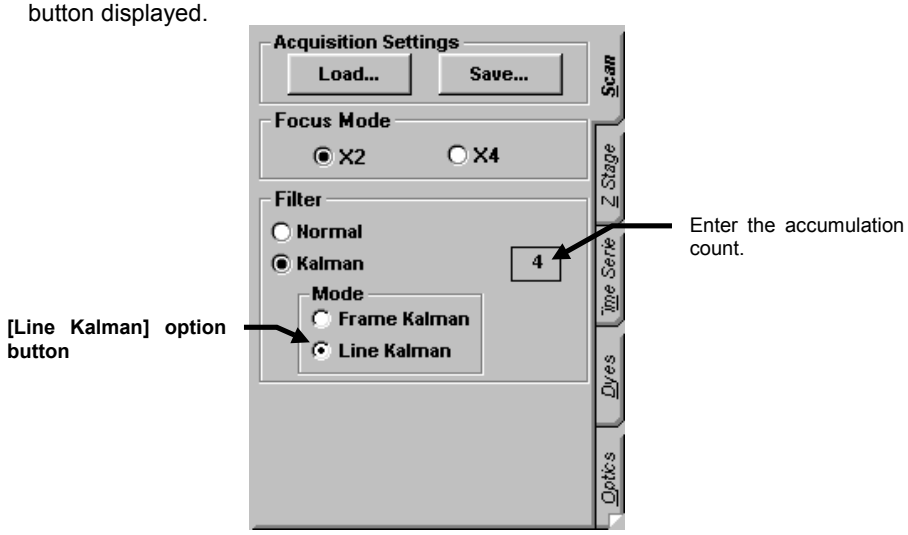


Fig. 2-12 [Scan] sub-panel

2. In the [Filter] group box, select the [Kalman] option button.

3. Enter the accumulation count in the text box and select the [Line Kalman] option button displayed.



TIP

The accumulation count can be set up a maximum of 63 times.
When 0 is set as the number of times of accumulation, an ordinary image acquisition is to be performed.

4. Click the <Once> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.
   And the accumulated image can be displayed in the [Display] panel without being displayed in full.

The difference between the Frame and Line modes of Kalman Accumulation

In the frame mode, an image is created by accumulation after scanning is performed per image at specified times. Meanwhile, in the line mode, scanning is performed per line at specified times and after accumulation is performed, the next line is to be scanned.
Each mode can be selected according to its ability; the frame mode is adequate for observing fixed sells and the line mode is adequate for observing living cells.

2-2-1-7 Saving the Acquired Image in File

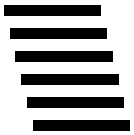
1. Display the [File I/O] panel.
2. Click the page tab of the [Live] panel showing the image to be saved, so that the image is displayed at the front.
3. Click the <Experiment> button in the [Save] group box in the [File I/O] panel.
   For details, see section 2-3-1, “Saving Image”.



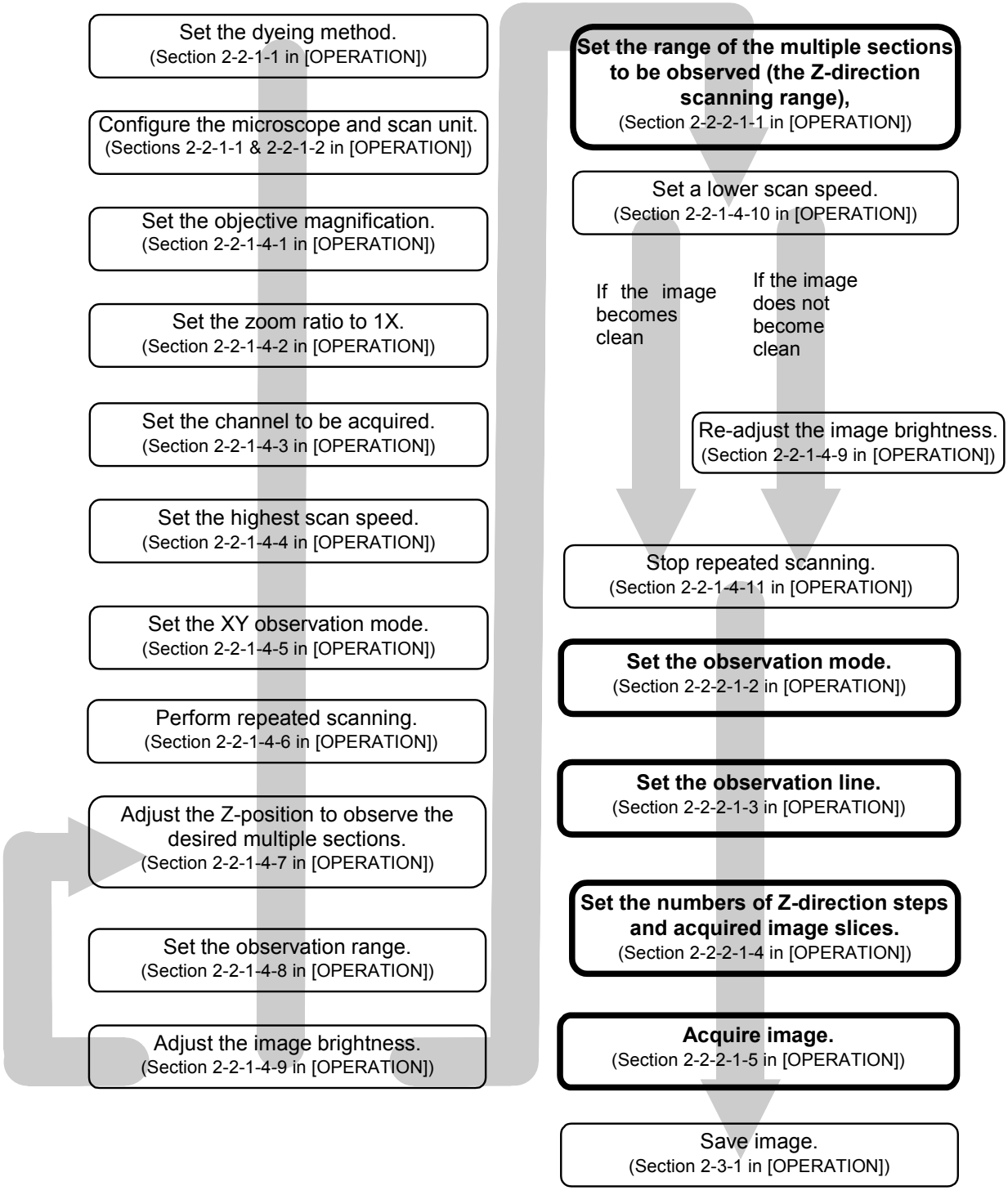
<Save> button

2-2-2 Image Acquisition in Other Observation Modes

2-2-2-1 XZ Observation Mode



The description in this section will be focused on the image acquisition operations in the XZ observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, “Image Acquisition in XY Observation Mode”. The details of each operation will be described in the subsequent sections.


Set the dyeing method.
(Section 2-2-1-1 in [OPERATION])
Configure the microscope and scan unit.
(Sections 2-2-1-1 & 2-2-1-2 in [OPERATION])
Set the objective magnification.
(Section 2-2-1-4-1 in [OPERATION])
Set the zoom ratio to 1X.
(Section 2-2-1-4-2 in [OPERATION])
Set the channel to be acquired.
(Section 2-2-1-4-3 in [OPERATION])
Set the highest scan speed.
(Section 2-2-1-4-4 in [OPERATION])
Set the XY observation mode.
(Section 2-2-1-4-5 in [OPERATION])
Perform repeated scanning.
(Section 2-2-1-4-6 in [OPERATION])
Adjust the Z-position to observe the desired multiple sections.
(Section 2-2-1-4-7 in [OPERATION])
Set the observation range.
(Section 2-2-1-4-8 in [OPERATION])
Adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Set the range of the multiple sections to be observed (the Z-direction scanning range),
(Section 2-2-2-1-1 in [OPERATION])
Set a lower scan speed.
(Section 2-2-1-4-10 in [OPERATION])
If the image becomes clean
If the image does not become clean
Re-adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Stop repeated scanning.
(Section 2-2-1-4-11 in [OPERATION])
Set the observation mode.
(Section 2-2-2-1-2 in [OPERATION])
Set the observation line.
(Section 2-2-2-1-3 in [OPERATION])
Set the numbers of Z-direction steps and acquired image slices.
(Section 2-2-2-1-4 in [OPERATION])
Acquire image.
(Section 2-2-2-1-5 in [OPERATION])
Save image.
(Section 2-3-1 in [OPERATION])

1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (i.e. the Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

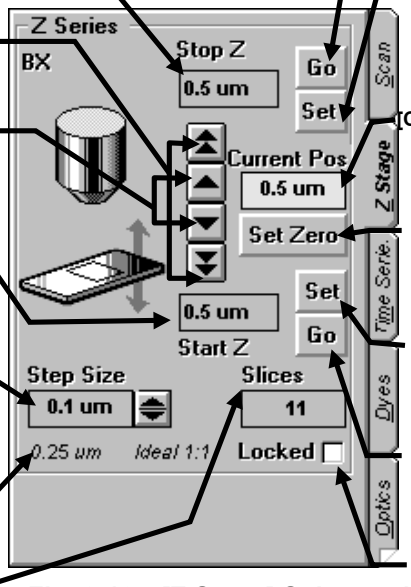
<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard.



<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Enables the Z-Motor operation.

Fig. 2-13 [Z Stage] Sub-panel

TIP

The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed.
See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.

Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).
4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

2 Setting the observation mode

1. In the [Scan Mode] group box in the [Acquire] panel, select the [Depth] option button.
2. In the [Acquire] panel, select the XZ observation mode option button.

3 Setting the observation line

1. A line is displayed on the image in the [Live] panel. Place the mouse pointer arrow on the line and drag it to the position you want to observe.

4 Setting the numbers of steps and acquired image slices

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.
2. The number of steps has already been set and displayed in the [Step Size] text box. This number can be changed using the <▲> or <▼> button in the [Step Size] text box.



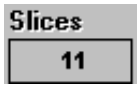
[Step Size] text box

TIP

The number of steps in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.

NOTE

The number of steps calculated by the system may be erroneous unless the XZ observation mode has been set previously.



[Slices] text box

TIP

The number of acquired images shown in the [Slices] text box can also be input from the keyboard.

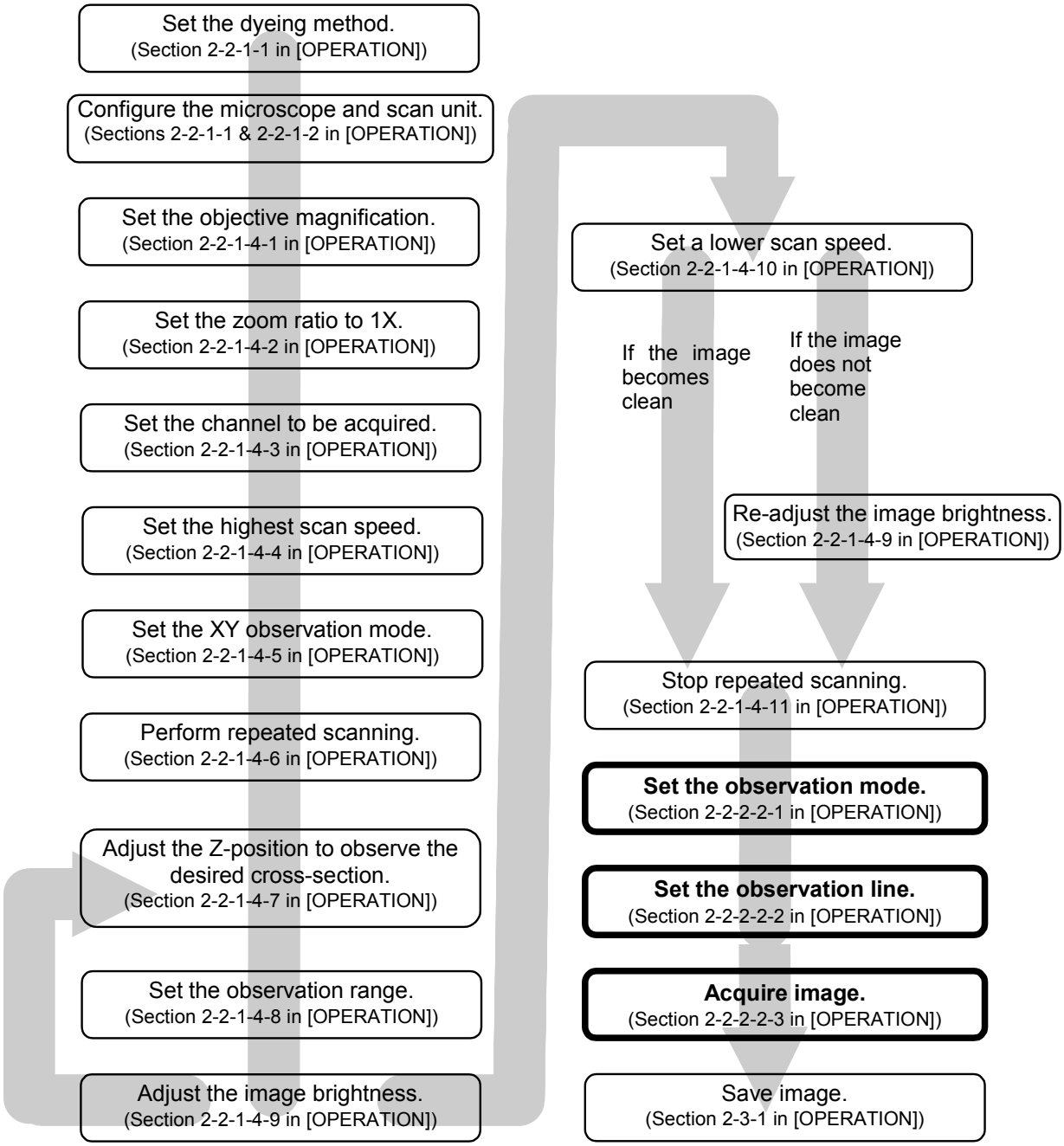
After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).

5 Acquiring image

1. Click the <XZ> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.

2-2-2-2 XT Observation Mode

The description in this section will be focused on the image acquisition operations in the XT observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, “Image Acquisition in XY Observation Mode”. The details of each operation will be described in the subsequent sections.



1 Setting the observation mode

1. In the [Scan Mode] group box in the [Acquire] panel, select the [Line] option button.
2. In the [Acquire] panel, select the XT observation mode option button.

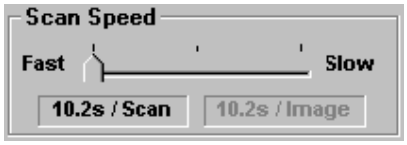
2 Setting the observation line

1. A line is displayed on the image in the [Live] panel. Place the mouse pointer arrow on the line and drag it to the position you want to observe.

3 Acquiring image

1. Click the <XT> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.

The XT observation acquires the images of the same position (line) for 2000 times successively at the set scanning speed. For reference, the time required for the image acquisitions is shown in the frame on the bottom left of the [Scan Speed] group box.



The number of image acquisitions (2000 times) can be changed by changing the value in the [N] text box in the [Time Series] sub-panel.

While acquiring an image in the XT observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.





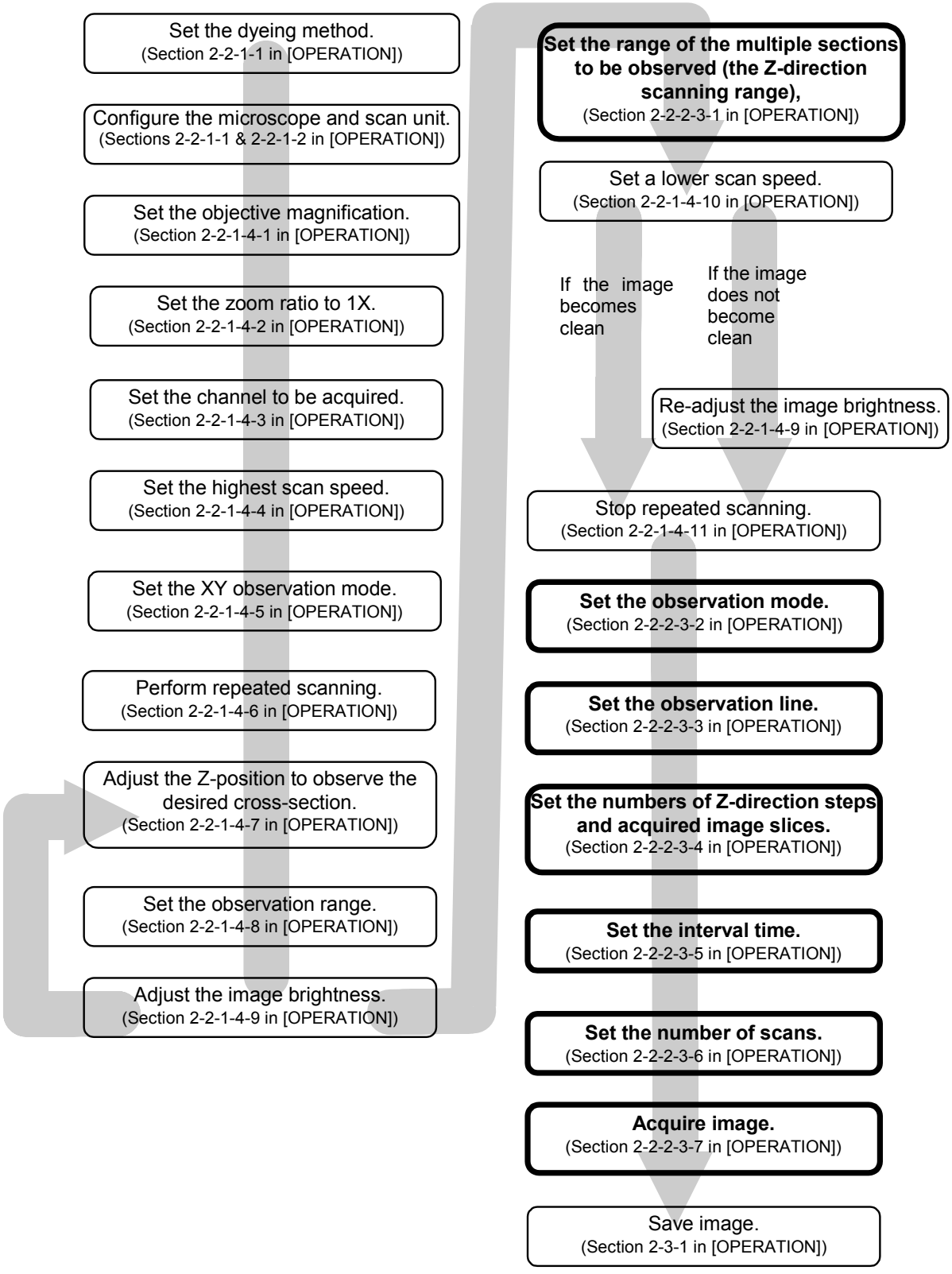
<Resume> button
Restarts image acquisition at the frame next to the frame where the acquisition is suspended.

<Series Done> button
Determines the acquired images. Once this button is clicked, it is not possible to append an image.

2-2-2-3 XZT Observation Mode



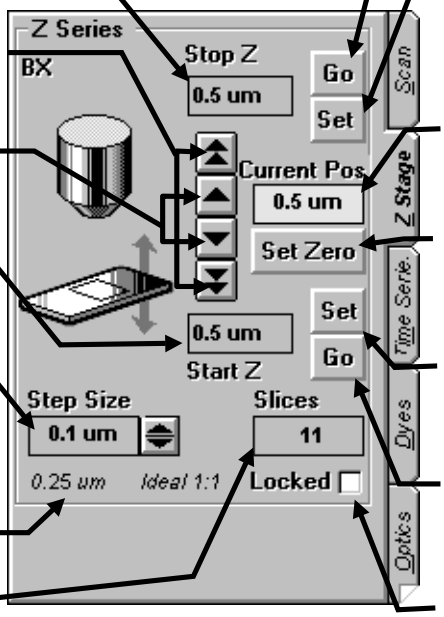
The description in this section will be focused on the image acquisition operations in the XZT observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, “Image Acquisition in XY Observation Mode”. The details of each operation will be described in the subsequent sections.


Set the dyeing method.
(Section 2-2-1-1 in [OPERATION])
Configure the microscope and scan unit.
(Sections 2-2-1-1 & 2-2-1-2 in [OPERATION])
Set the objective magnification.
(Section 2-2-1-4-1 in [OPERATION])
Set the zoom ratio to 1X.
(Section 2-2-1-4-2 in [OPERATION])
Set the channel to be acquired.
(Section 2-2-1-4-3 in [OPERATION])
Set the highest scan speed.
(Section 2-2-1-4-4 in [OPERATION])
Set the XY observation mode.
(Section 2-2-1-4-5 in [OPERATION])
Perform repeated scanning.
(Section 2-2-1-4-6 in [OPERATION])
Adjust the Z-position to observe the desired cross-section.
(Section 2-2-1-4-7 in [OPERATION])
Set the observation range.
(Section 2-2-1-4-8 in [OPERATION])
Adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Set the range of the multiple sections to be observed (the Z-direction scanning range),
(Section 2-2-2-3-1 in [OPERATION])
Set a lower scan speed.
(Section 2-2-1-4-10 in [OPERATION])
If the image becomes clean
If the image does not become clean
Re-adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Stop repeated scanning.
(Section 2-2-1-4-11 in [OPERATION])
Set the observation mode.
(Section 2-2-2-3-2 in [OPERATION])
Set the observation line.
(Section 2-2-2-3-3 in [OPERATION])
Set the numbers of Z-direction steps and acquired image slices.
(Section 2-2-2-3-4 in [OPERATION])
Set the interval time.
(Section 2-2-2-3-5 in [OPERATION])
Set the number of scans.
(Section 2-2-2-3-6 in [OPERATION])
Acquire image.
(Section 2-2-2-3-7 in [OPERATION])
Save image.
(Section 2-3-1 in [OPERATION])

1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.



[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard.

<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Enables the Z-Motor operation.

Fig. 2-14 [Z Stage] Sub-panel

TIP

The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed.
See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.

Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).

4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

2 Setting the observation mode

1. In the [Scan Mode] group box in the [Acquire] panel, select the [Depth] option button.

2. In the [Acquire] panel, select the XZT observation mode option button.

3 Setting the observation line

1. A line is displayed on the image in the [Live] panel. Place the mouse pointer arrow on the line and drag it to the position you want to observe.

4 Setting the numbers of steps and acquired image slices

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

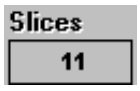


[Step Size] text box

2. A number of steps is displayed in the [Step Size] text box. This number can be changed using the <▲> or <▼> button in the [Step Size] text box.

TIP

The number of steps shown in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.



[Slices] text box

NOTE

The number of steps calculated by the system may be erroneous unless the XZT observation mode has been set previously.

TIP

The number of acquired images shown in the [Slices] text box can also be input from the keyboard.

After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).

5 Setting the interval time

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel.
2. Set the interval time using the <▲> or <▼> button in the [Interval] text box.

[Interval] text box
Set the interval time using the <▲> or <▼> button or by input from the keyboard.

[N] text box
Set the number of scans using the <▲> or <▼> button or by input from the keyboard.

[Rest Time] text box
Shows the time after end of acquisition of an image until the start of next acquisition.

[Total Time] text box
Shows the total time required for image

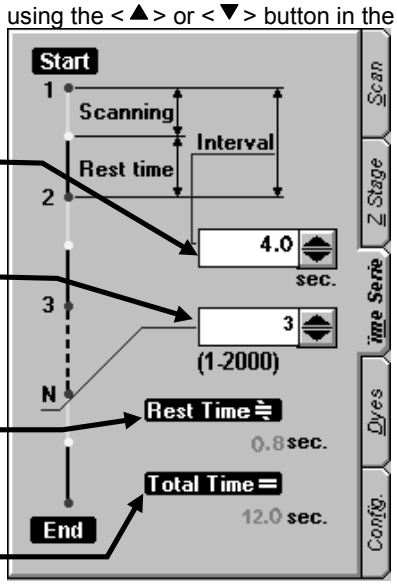


Fig. 2-15 [Time Series] Sub-panel

TIP

An image with no interval can be acquired by entering [0] in the [Interval] text box.
In this case, the [Interval] text box shows “Free Run” message.
Using <▼> button also sets the [Interval] text box to “Free Run”.

6 Setting the number of scans

1. Set the number of scans using the <▲> or <▼> button in the [N] text box in the [Time Series] sub-panel.

7 Acquiring image

1. Click the <XZT> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.

While acquiring an image in the XZT observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.



<Resume> button
Restarts image acquisition at the frame next to the frame where the acquisition is suspended.



<Series Done> button
Determines the acquired images. Once this button is clicked, it is not possible to append an image.

8 Appending image

An additional image can be appended to the image acquired in the XZT observation mode.
Immediately after acquisition of an image in the XZT observation mode, the buttons at the upper part of the [Acquire] panel changes as shown below.

<Series Done> button
Determines the acquired images. Once this button is clicked, it is not possible to append an image.

<Append Next> button
Acquires another image and appends it to the image acquired immediately before.

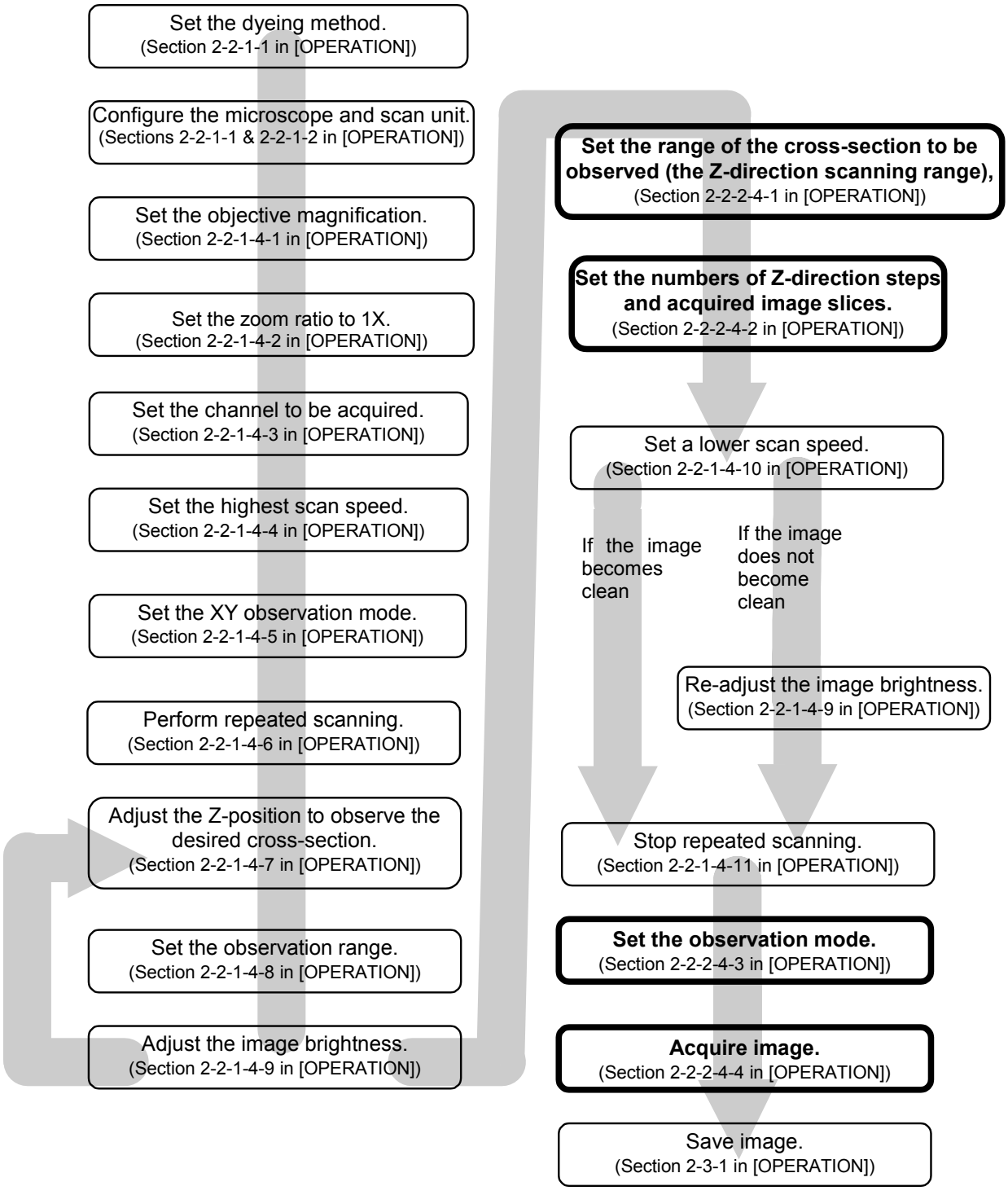


Click the <Append Next> button to append an image. An image will be acquired with the same number of steps as the image acquired immediately before and appended to it.
Click the <Series Done> button when it is not required to append an image.

2-2-2-4 XYZ Observation Mode



The description in this section will be focused on the image acquisition operations in the XYZ observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, “Image Acquisition in XY Observation Mode”. The details of each operation will be described in the subsequent sections.


Set the dyeing method.
(Section 2-2-1-1 in [OPERATION])
Configure the microscope and scan unit.
(Sections 2-2-1-1 & 2-2-1-2 in [OPERATION])
Set the objective magnification.
(Section 2-2-1-4-1 in [OPERATION])
Set the zoom ratio to 1X.
(Section 2-2-1-4-2 in [OPERATION])
Set the channel to be acquired.
(Section 2-2-1-4-3 in [OPERATION])
Set the highest scan speed.
(Section 2-2-1-4-4 in [OPERATION])
Set the XY observation mode.
(Section 2-2-1-4-5 in [OPERATION])
Perform repeated scanning.
(Section 2-2-1-4-6 in [OPERATION])
Adjust the Z-position to observe the desired cross-section.
(Section 2-2-1-4-7 in [OPERATION])
Set the observation range.
(Section 2-2-1-4-8 in [OPERATION])
Adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Set the range of the cross-section to be observed (the Z-direction scanning range),
(Section 2-2-2-4-1 in [OPERATION])
Set the numbers of Z-direction steps and acquired image slices.
(Section 2-2-2-4-2 in [OPERATION])
Set a lower scan speed.
(Section 2-2-1-4-10 in [OPERATION])
If the image becomes clean
If the image does not become clean
Re-adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Stop repeated scanning.
(Section 2-2-1-4-11 in [OPERATION])
Set the observation mode.
(Section 2-2-2-4-3 in [OPERATION])
Acquire image.
(Section 2-2-2-4-4 in [OPERATION])
Save image.
(Section 2-3-1 in [OPERATION])

1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard.

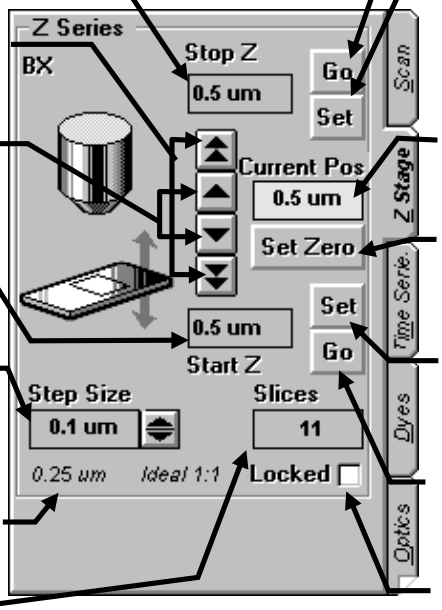


Fig. 2-16 [Z Stage] Sub-panel

<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Enables the Z-Motor operation.

TIP

The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed.
See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.

Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).

4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

2 Setting the numbers of steps and acquired image slices

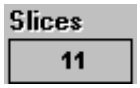


[Step Size] text box

1. Set the number of steps using the <▲> or <▼> button in the [Step Size] text box.

TIP

The number of steps shown in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.



[Slices] text box

TIP

The number of acquired images shown in the [Slices] text box can also be input from the keyboard.
After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).

3 Setting the observation mode

1. In the [Scan Mode] group box in the [Acquire] panel, select the [Surface] option button.
2. In the [Acquire] panel, select the XYZ observation mode option button.

4 Acquiring image

1. Click the <XYZ> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.

While acquiring an image in the XYZ observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.





<Resume> button

Restarts image acquisition at the frame next to the frame where the acquisition is suspended.

<Series Done> button

Determines the acquired images. Once this button is clicked, it is not possible to append an image.

5 Appending image

An additional image can be appended to an image acquired in the XYZ observation mode.

Immediately after acquisition of an image in the XYZ observation mode, the buttons at the upper part of the [Acquire] panel changes as shown below.

<Append Next> button

Acquires another image and appends it to the image acquired immediately before.



<Series Done> button

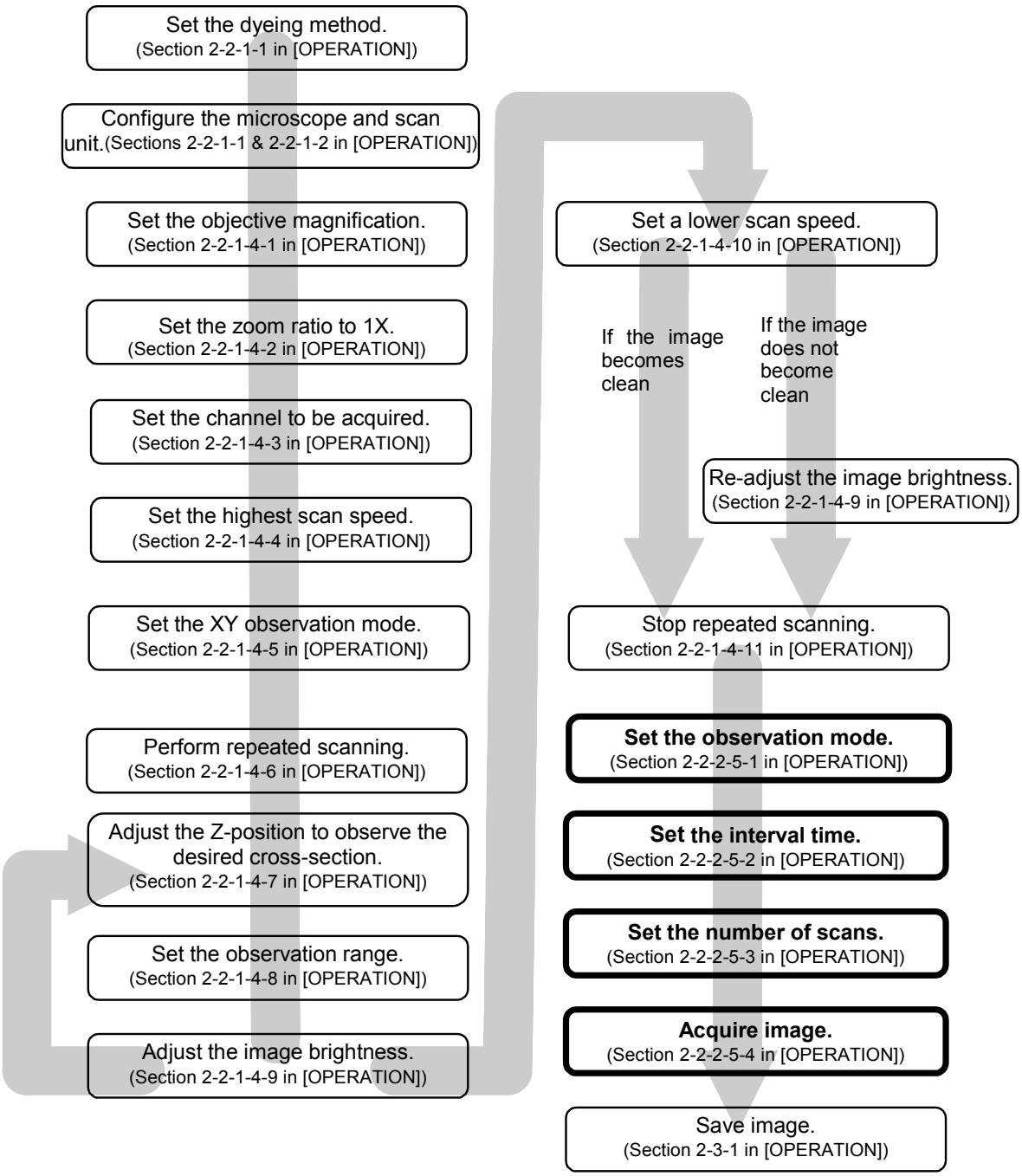
Determines the acquired images. Once this button is clicked, it is not possible to append an image.

Click the <Append Next> button to append an image. An image will be acquired with the same number of steps as the image acquired immediately before and appended to it.

Click the <Series Done> button when it is not required to append an image.

2-2-2-5 XYT Observation Mode

The description in this section will be focused on the image acquisition operations in the XYT observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, “Image Acquisition in XY Observation Mode”. The details of each operation will be described in the subsequent sections.



1 Setting the observation mode

1. In the [Scan Mode] group box in the [Acquire] panel, select the [Surface] option button.

2. In the [Acquire] panel, select the XYT observation mode option button.

2 Setting the interval time

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel. A panel as shown below will be displayed.

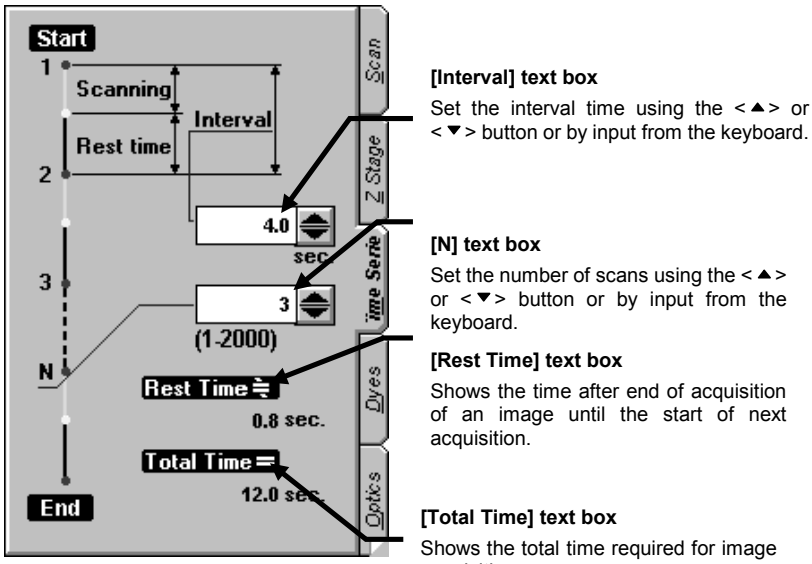


Fig. 2-17 [Time Series] Sub-panel

2. Set the interval time using the < ▲ > or < ▼ > button in the [Interval] text box.

TIP

An image with no interval can be acquired by entering [0] in the [Interval] text box.

In this case, the [Interval] text box shows “Free Run” message.

Using < ▼ > button also sets the [Interval] text box to “Free Run”.

3 Setting the number of scans

1. Set the number of scans using the <▲> or <▼> button in the [N] text box in the [Time Series] sub-panel.

4 Acquiring image

Click the <XYT> button in the [Acquire] panel.

The acquired image will be displayed in the [Live] panel.

When the built-in power supply for transmitted light illumination is used for a long period in the XYT mode, the metallic parts may be expanded by heat, causing the focusing to be deviated. To acquire precise image data, it is recommended to set the power switch of the transmitted light illumination to “O” (OFF).

While acquiring an image in the XYT observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.



<Resume> button

Restarts image acquisition at the frame next to the frame where the acquisition is suspended.



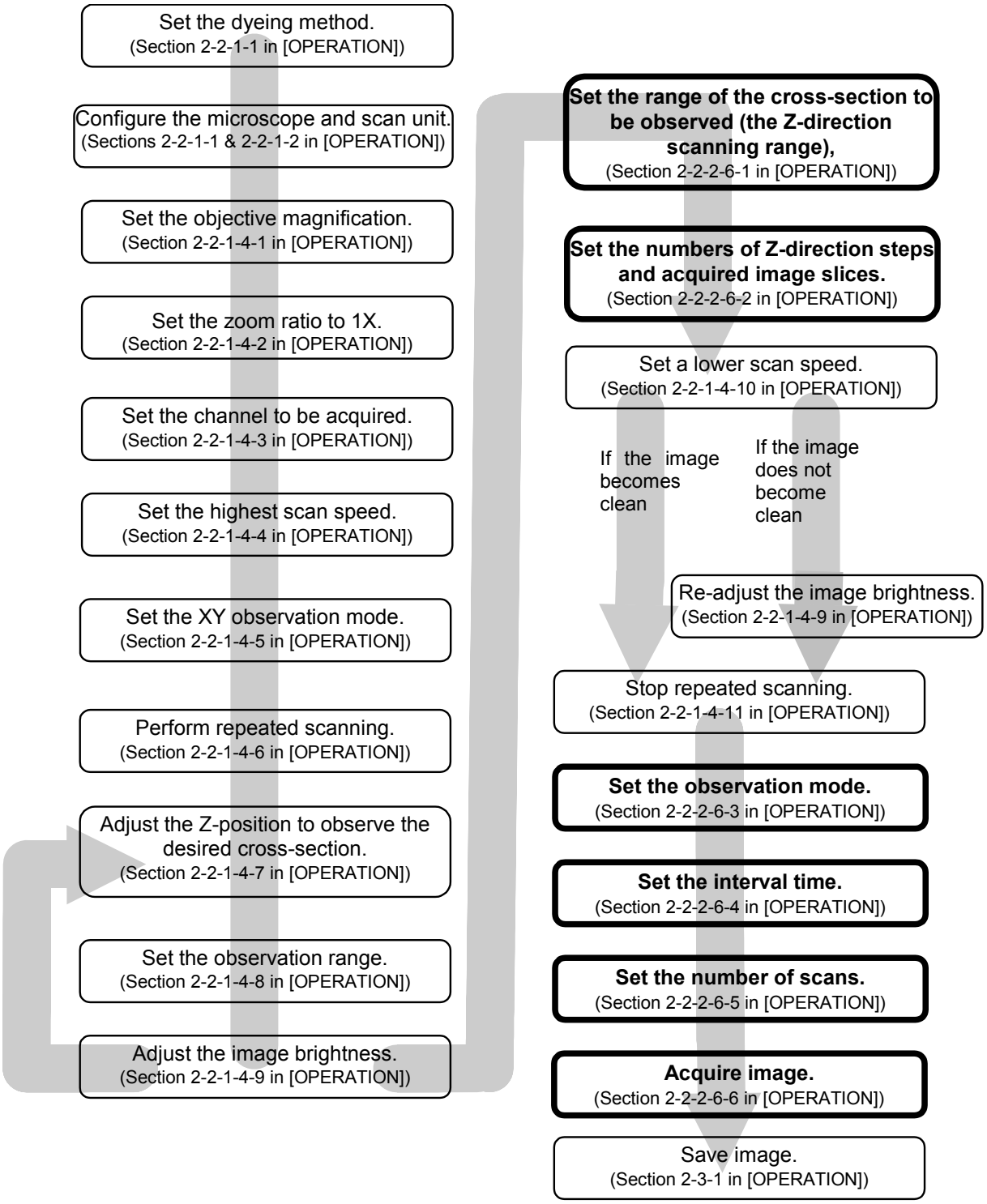
<Series Done> button

Determines the acquired images. Once this button is clicked, it is not possible to append an image.

2-2-2-6 XYZT Observation Mode



The description in this section will be focused on the image acquisition operations in the XYZT observation mode that are not used in the XY observation modes (which are the operations enclosed in ▭ in the chart on the next page). For other operations, see section 2-2-1, “Image Acquisition in XY Observation Mode”. The details of each operation will be described in the subsequent sections.


Set the dyeing method.
(Section 2-2-1-1 in [OPERATION])
Configure the microscope and scan unit.
(Sections 2-2-1-1 & 2-2-1-2 in [OPERATION])
Set the objective magnification.
(Section 2-2-1-4-1 in [OPERATION])
Set the zoom ratio to 1X.
(Section 2-2-1-4-2 in [OPERATION])
Set the channel to be acquired.
(Section 2-2-1-4-3 in [OPERATION])
Set the highest scan speed.
(Section 2-2-1-4-4 in [OPERATION])
Set the XY observation mode.
(Section 2-2-1-4-5 in [OPERATION])
Perform repeated scanning.
(Section 2-2-1-4-6 in [OPERATION])
Adjust the Z-position to observe the desired cross-section.
(Section 2-2-1-4-7 in [OPERATION])
Set the observation range.
(Section 2-2-1-4-8 in [OPERATION])
Adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Set the range of the cross-section to be observed (the Z-direction scanning range),
(Section 2-2-2-6-1 in [OPERATION])
Set the numbers of Z-direction steps and acquired image slices.
(Section 2-2-2-6-2 in [OPERATION])
Set a lower scan speed.
(Section 2-2-1-4-10 in [OPERATION])
If the image becomes clean
If the image does not become clean
Re-adjust the image brightness.
(Section 2-2-1-4-9 in [OPERATION])
Stop repeated scanning.
(Section 2-2-1-4-11 in [OPERATION])
Set the observation mode.
(Section 2-2-2-6-3 in [OPERATION])
Set the interval time.
(Section 2-2-2-6-4 in [OPERATION])
Set the number of scans.
(Section 2-2-2-6-5 in [OPERATION])
Acquire image.
(Section 2-2-2-6-6 in [OPERATION])
Save image.
(Section 2-3-1 in [OPERATION])

1 Setting the Z-direction scanning range

While acquiring image, move the Z stage according to the range of the multiple sections to be observed (Z-direction scanning range).

From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel.

[Stop Z] text box
Shows the scan stop position in the range of the observed cross-section (Z-direction scanning range).

<Z stage coarse adjustment> buttons
Displaces the Z stage on a large scale.

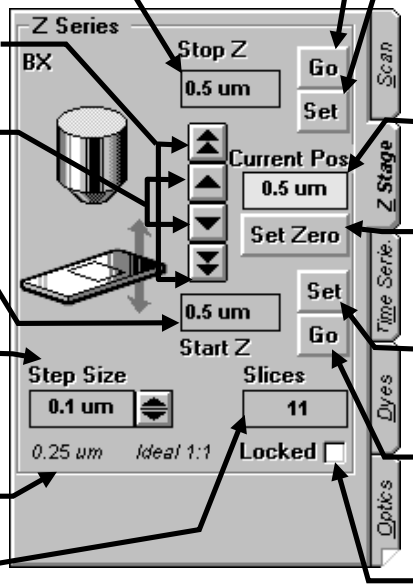
<Z stage fine adjustment> buttons
Displaces the Z stage on a fine scale.

[Start Z] text box
Shows the scan start position in the range of the observed cross-section (Z-direction scanning range).

[Step Size] text box
Set the number of steps using the <▲> or <▼> button. This number can also be input directly from the keyboard.

Recommended step size
Shows the number of steps calculated by the system so that the scale of depth in the Z-direction of the acquired image is identical to the scale of the plane in the X- and Y-directions.

[Slices] text box
Shows the number of images acquired. This number can also be input directly from the keyboard.



<Go> button
Moves to the set scanning stop position. Use this button to check the scanning stop position.

<Set> button
Sets the current stage position as the scanning stop position of the range of the observed cross-section (Z-direction scanning range).

[Current Pos] text box
Shows the current position of the stage.

<Set Zero> button
Sets the current stage position as the home position. Pressing this button also clears the [Stop Z] and [Start Z] values.

<Set> button
Sets the current stage position as the scanning start position of the range of the observed cross-section (Z-direction scanning range).

<Go> button
Moves to the set scan start position. Use this button to check the scan start position.

[Locked] check box
Enables the Z-Motor operation.

Fig. 2-18 [Z Stage] Sub-panel

TIP

The moving amount assigned to the <Z stage fine adjustment> and <Z stage coarse adjustment> buttons can be changed.
See section 1-3 in MAINTENANCE, “Setting the System Configuration” for detailed operations.

1. Check the [Locked] check box in the [Z Stage] sub-panel.

Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.

2. While observing the image in the [Live] panel, locate the upper edge of the range to be observed by moving down the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the bottom edge of the range to be observed by moving down the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

3. When the upper edge position is located, click the <Set> button. The [Start Z] text box will show the scan start position of the range of the multiple sections to be observed (Z-direction scanning range).
4. While observing the image in the [Live] panel, locate the bottom edge of the range to be observed by moving up the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel.

   When using the FLUOVIEW system with an inverted microscope, locate the upper edge of the range to be observed by moving up the revolving nosepiece using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons.

5. When the position is located, click the <Set> button. The [Stop Z] text box will show the scanning stop position of the range of the multiple sections to be observed (Z-direction scanning range).

2 Setting the numbers of steps and acquired image slices

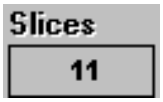


[Step Size] text box

1. Set the number of steps using the < ▲ > or < ▼ > button in the [Step Size] text box.

TIP

The number of steps shown in the [Step Size] text box has been calculated by the system so that the depth scale of the acquired image is identical to the horizontal scale.



[Slices] text box

TIP

The number of acquired images shown in the [Slices] text box can also be input from the keyboard.
After setting [Start Z] (Z-direction scan start position) and [Stop Z] (Z-direction scanning stop position), input the desired number of images in the [Slices] text box. This automatically sets [Step Size] (number of steps).

3 Setting the observation mode

1. In the [Scan Mode] group box in the [Acquire] panel, select the [Surface] option button.
2. In the [Acquire] panel, select the XYZT observation mode option button.

4 Setting the interval time

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel. A panel as shown below will be displayed.

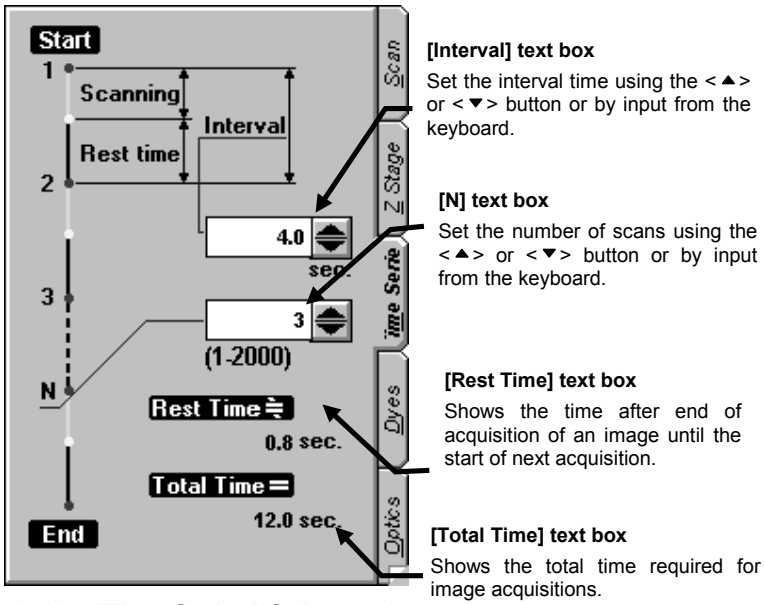


Fig. 2-19 [Time Series] Sub-panel

2. Set the interval time using the < ▲ > or < ▼ > button in the [Interval] text box.

TIP

An image with no interval can be acquired by entering [0] in the [Interval] text box.
In this case, the [Interval] text box shows “Free Run” message.
Using < ▼ > button also sets the [Interval] text box to “Free Run”.

5 Setting the number of scans

1. Set the number of scans using the <▲> or <▼> button in the [N] text box in the [Time Series] sub-panel.

6 Acquiring image

1. Click the <XYZT> button in the [Acquire] panel.
   The acquired image will be displayed in the [Live] panel.
   When the built-in power supply for transmitted light illumination is used for a long period in the XYZT mode, the metallic parts may be expanded by heat, causing the focusing to be deviated. To acquire precise image data, it is recommended to turn off the power of the transmitted light.

While acquiring an image in the XYZT observation mode, clicking the <STOP SCAN> button changes the buttons at the upper part of the [Acquire] panel as shown below. The <Resume> button restarts image acquisition at the frame next to the frame where the acquisition is suspended.





<Resume> button
Restarts image acquisition at the frame next to the frame where the acquisition is suspended.

<Series Done> button
Determines the acquired images. Once this button is clicked, it is not possible to append an image.

2-2-3 Differences in Image Acquisition Method Between Fluorescent and Transmitted Images

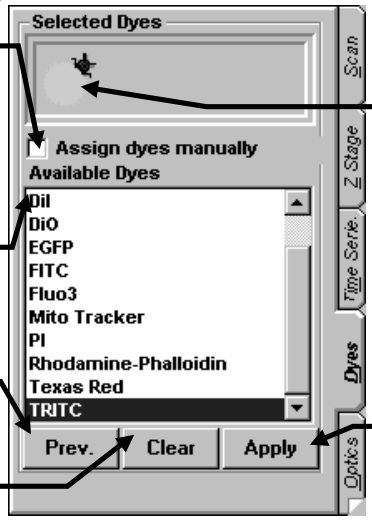
2-2-3-1 Monochrome Image

The wavelength obtained by monochrome dyeing can be acquired and observed as an image of a channel (Ch1/Ch2/Ch3/Ch4).

The subsequent description deals with the differences in operation between the monochrome dyeing and dual-fluorochrome dyeing.

- Set the dyeing method

  1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.



[Assign dyes manually] check box
Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

[Available Dyes] list box
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Prev.> button
Sets the dyeing method which was set last time by clicking the <Apply> button.

<Clear> button
Clear the set dyeing method.

Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

<Apply> button
Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.

Fig. 2-20 [Dyes] Sub-panel

  2. Select the specimen dyeing method in the [Available Dyes] list box in the [Selected Dyes] group box, and drag the selected method into the field immediately above the list box.

  3. Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

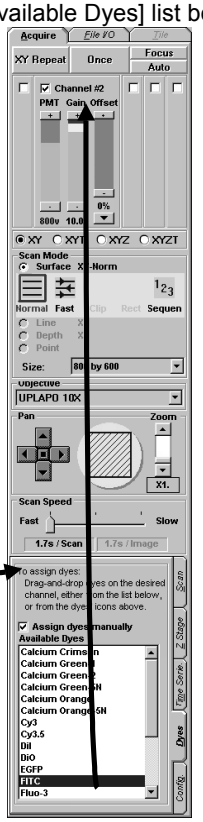
TIP

When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the changed filter. And the dyeing method is shown in the [Ch] group box.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

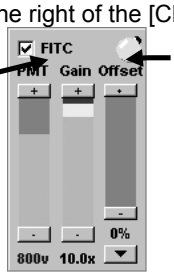
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set

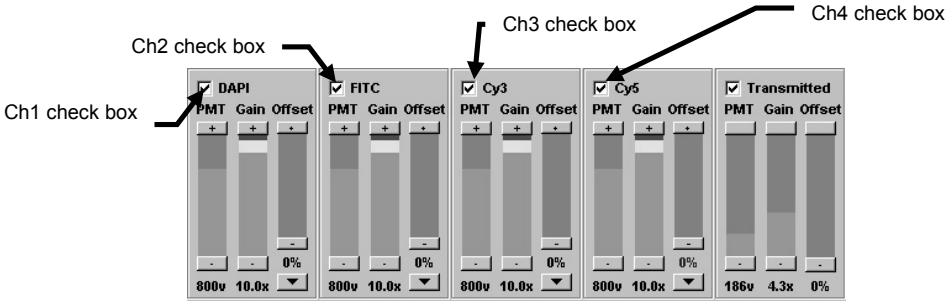


Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

- With types of dyeing of the specimen to observe, the barrier filter will be set automatically to the optical path.
  To change the barrier filter types, see section 1-3-2-4, “Configuring the Filters” and change by [Optical System Configuration] window.

- Make the channel ready for image acquisition.
  In the [Acquire] panel, make sure that the check box in the [Ch1]/[Ch2]/[Ch3]/[Ch4] group box that shows the dyeing method is check-marked to indicate that the corresponding channel is ready for image acquisition.



- Set the image brightness.
  Adjust the brightness of the image of the image acquisition channel by using the [PMT], [Offset] and [Gain] LED sliders in the [Acquire] panel. For details, see to section 2-2-1-4-9, “Adjusting the Image Brightness” in the [OPERATION] volume.

2-2-3-2 Dual-Fluorochrome Image

The wavelength obtained by dual-fluorochrome dyeing can be acquired and observed as images of the channels (Ch1 or Ch2).

The subsequent description deals with the differences in operation between the monochrome dyeing and dual-fluorochrome dyeing.

- Set the two or more dyeing methods.

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

[Assign dyes manually] check box

Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

[Available Dyes] list box

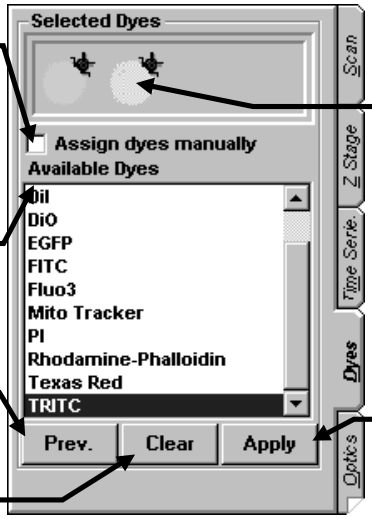
Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Prev.> button

Sets the dyeing method which was set last time by clicking the <Apply> button.

<Clear> button

Clear the set dyeing method.



Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

<Apply> button

Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.

Fig. 2-21 [Dyes] Sub-panel

2. Select the specimen dyeing method by dragging desired dye names in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

3. Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

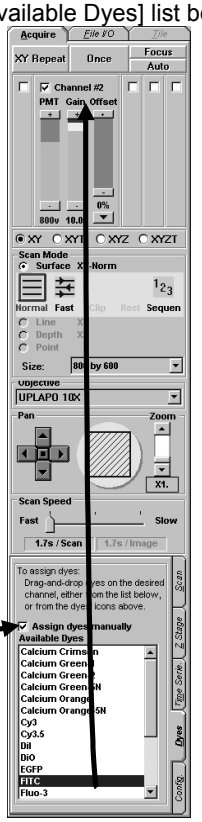
TIP

When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the changed filter. And the dyeing method is shown in the [Ch] group box.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

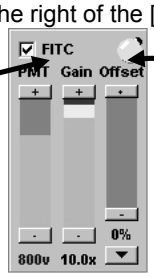
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set

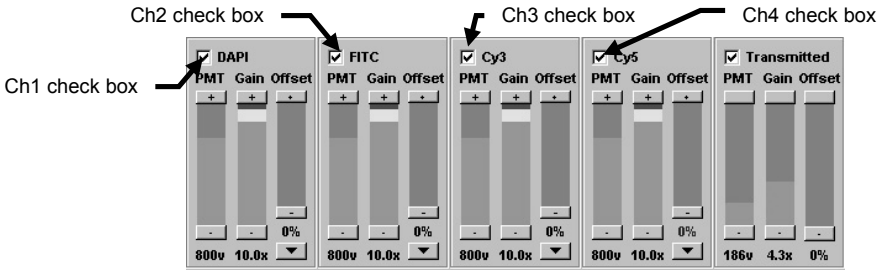


Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

- With types of dyeing of the specimen to observe, the Barrier Filters will be set automatically to the optical path.
  To change the Barrier Filter types, see to section 1-3-2-4, “Configuring the Filters” and change by [Optical System Configuration] window.

- Make the channels ready for image acquisition.
  In the [Acquire] panel, make sure that the check box in the [Ch1]/[Ch2]/[Ch3]/[Ch4] group boxes that show the dyeing methods are check-marked to indicate that the corresponding channels are ready for image acquisition.



- When observing a fluorescence image(s) simultaneously, also set the brightness of the fluorescence image(s).
  Adjust the brightness of the image of each channel by using the [PMT], [Offset] and [Gain] LED sliders in the [Acquire] panel. For details, see section 2-2-1-4-9, “Adjusting the Image Brightness”.

2-2-3-3 Transmitted Image

Images obtained by transmitted light observation can also be acquired or observed simultaneously with images obtained by fluorescence observation.

- When observing fluorescence images simultaneously, set the dyeing method.

  1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

[Assign dyes manually] check box

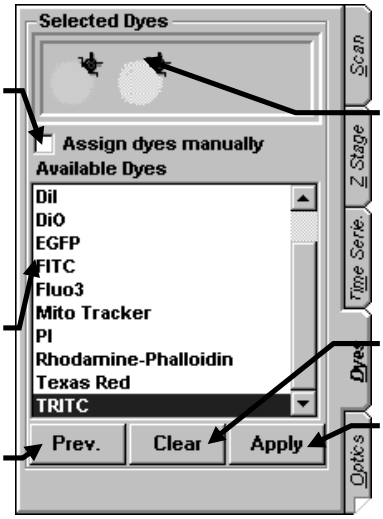
Checking this enables the manual setting. Dragging the dyeing method in the list directly to the [Ch] group box assigns the dye to the desired channel.

[Available Dyes] list box

Lists the available dyes. Select the desired items from this list and drag them to the field above it to select the dyeing method.

<Prev.> button

Sets the dyeing method which was set last time by clicking the <Apply> button.



Place the pointer on the icon displayed in the [Selected Dyes], and the dyeing method is shown in the pop-up display.

<Clear> button

Clear the set dyeing method.

<Apply> button

Applies the dyeing method dragged in the [Selected Dyes] group box to the [Ch] group in the [Acquire] panel.

Fig. 2-22 [Dyes] Sub-panel

  2. Select the specimen dyeing method by dragging desired dye names in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

  3. Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

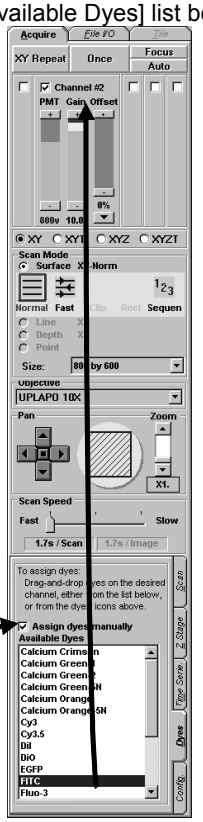
TIP

When the dyeing method is selected from the [Available Dyes] list box and the <Apply> button is clicked, a channel for acquiring fluorescence is set automatically according to the changed filter. And the dyeing method is shown in the [Ch] group box.

One Point!

The [Assign dyes manually] check box can also be used to set the dyeing method to the desired channel.

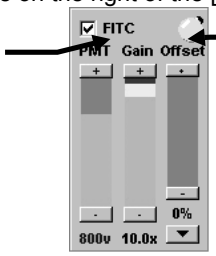
1. Check the [Assign dyes manually] check box in the [Dyes] sub-panel.
2. Select the dyeing method in the [Available Dyes] list box and drag it directly to the field of the [Ch] check box.



[Assign dyes manually] check box

3. After dragging, the icon appears on the right of the [Ch] check box and the dyeing method is set.

The dyeing method is set



Icon

Dragging the icon to the out of the [Ch] check box field cancels the setting of the dyeing method.

- With types of dyeing of the specimen to observe, the Barrier Filters will be set automatically to the optical path.
  To change the Barrier Filter types, see section 1-3-2-4,"Configuring the Filters " and change by [Optical system Configuration] window.

- Turn off the transmitted light bulb of the microscope.
  1. From the page tabs on the bottom right of the [Acquire] panel, select the [Optics] sub-panel.

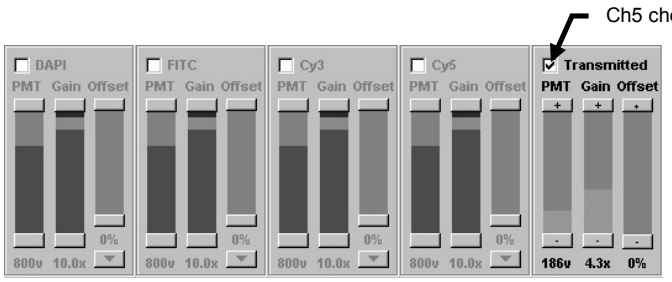


<Trans. Lamp> button
Turns off the transmitted light bulb of the microscope.
(The lamp lights when the button is in the pressed status.)

Fig. 2-23 [Optics] Sub-panel

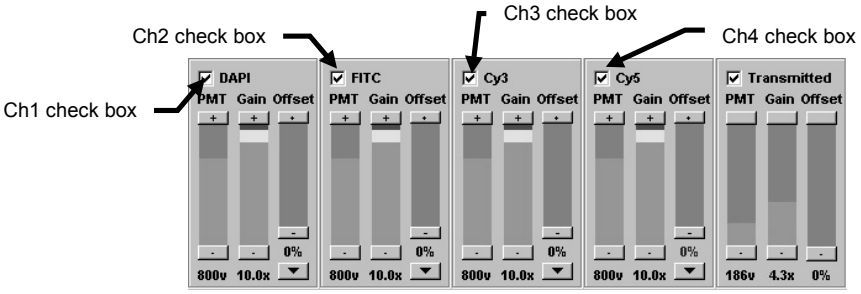
  2. Click the <Trans. Lamp> button to turn the lamp off (set the button to the non-pressed status).

- In the [Acquire] panel, make sure that the [Transmitted] check box in the [Ch5] group box is check-marked to indicate that Ch3 is ready for image acquisition.



- When observing a fluorescence image simultaneously, set the required channel ready for acquisition of fluorescence image.

  Make sure that the check box showing the dyeing method in the [Ch1]/ [Ch2]/[Ch3]/[Ch4] group box is check-marked to indicate that the corresponding channel is ready for image acquisition.



- Set the brightness of the transmitted image.

  Please use PMT, Offset and Gain LED sliders in [Ch5] group box to adjust the brightness of the transmitted image. For details, see section 2-2-1-4-9, “Adjusting the Image Brightness” in the [OPERATION] volume.

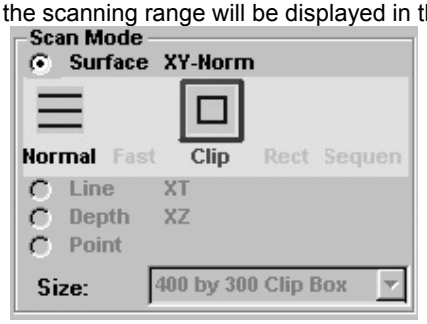
- When observing a fluorescence image(s) simultaneously, also set the brightness of the fluorescence image(s).

  Adjust the brightness of the image of the transmitted image acquisition channel(s) by using the [PMT], [Offset] and [Gain] LED sliders in its group box in the [Acquire] panel. For details, see section 2-2-1-4-9, “Adjusting the Image Brightness” in the [OPERATION] volume.

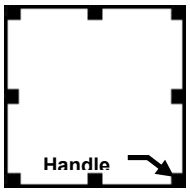
2-2-4 Acquiring Multiple Images (XYZ, XYT or XYZT Observation)

Acquisition of a large number of image slices takes a very long time. In such a case, it is possible to acquire the images of only the required areas in a shorter time by narrowing the observation range and scanning range.

1. Acquire an image in the XY observation mode. For the detailed operation method, see section 2-2-1, “Image Acquisition in XY Observation Mode”.
2. Select the [Surface XY-Norm] option button in the [Scan Mode] group box in the [Acquire] panel, then select <Clip> from the list displayed below it.
   A frame indicating the scanning range will be displayed in the [Live] panel.



3. Move the frame around the range to be observed. The frame can be moved by placing the mouse pointer inside it and dragging.
4. Change the frame size. To change the frame size, click a point inside the frame with the mouse pointer. When square handles are displayed on the frame edges, place the mouse pointer on one of them and drag it.



5. Acquire images by clicking the <XYZ>, <XYT> or <XYZT> button.
6. After acquiring images, select <Normal> from the list displayed below the [Surface XY-Norm] option button in the [Scan Mode] group box to set the scanning range to the original setting.

NOTE

It is not permitted to change the scanning range after having acquired images. If you want to change the scanning range again, select <Normal> from the list displayed below the [Surface XY-Norm] option button in the [Scan Mode] group box to set the scanning ragne to the original setting, acquire an image in the XY observation mode, then select <Clip> in the [Scan Mode] group box and reselect the scanning range.

2-2-5 High-Speed Image Acquisition

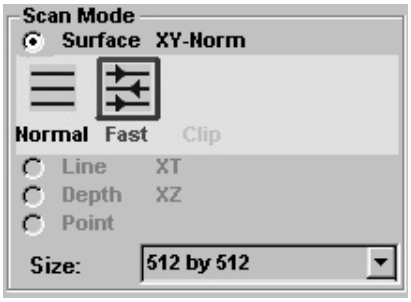
An image can be acquired in 0.25 second.

This image acquisition mode is valid under the following condition:

- Max. 2 channels
- Image size: 512 x 512 pixels
- Zoom ratio of 2X or more, with setting in 2X steps

1. Check one or both of the [Ch] check boxes in the [Ch] group box in the [Acquire] panel.

2. Select the [Surface XY-None] option button in the [Scan Mode] group box in the [Acquire] panel. Then select <Fast> in the list shown below.



[512 by 512] is set automatically in the [Size] drop-down list in the [Scan Mode] group box.

"X2" is set and shown in gray-out display automatically in the [Zoom] scale in the [Pan] group box.

3. To change the zoom ratio, use the [Zoom] scale in the [Pan] group box.



<XY Repeat> button

4. Select any button to acquire image such as the <XY Repeat> button.

TIP High-speed image acquisition is available in the XY, XYT, XT observations.



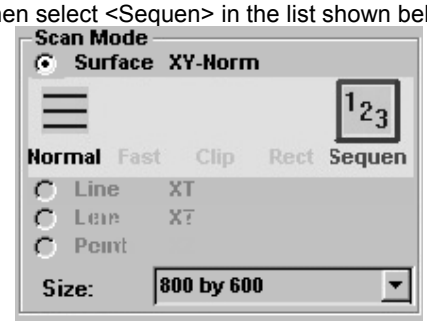
<Once> button

5. After acquiring images, select <Normal> from the list displayed below the [Surface XY-Norm] option button in the [Scan Mode] group box to set the scanning range to the original setting.

2-2-6 Acquiring Finer Image (Sequential Scan)

An image without containing superimposition of fluorescence can be obtained by acquiring the image slice of each laser excitation or channel sequentially.
With this image capturing method, the image of a multiple-dyed specimen can be obtained by sequentially acquiring image slice of each type of fluorescence.

1. Select the [Surface XY-Note] option button in the [Scan Mode] group box in the [Acquire] panel, then select <Sequen> in the list shown below.



Selecting <Sq Clp>, <Sq Rct>, or <Line Sq> enables the image acquisition of the desired rectangle area or that of the rectangle area at desired angle, or a line sequential scan..

Line Sequential Scan

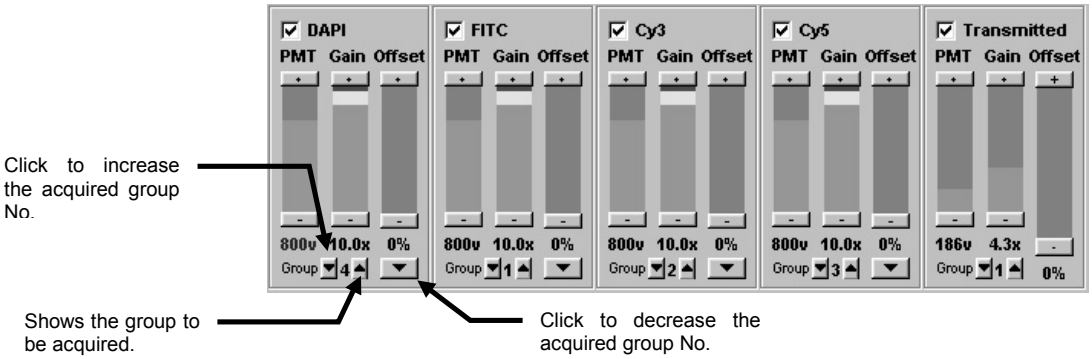
Ordinary sequential scan is performed for every one frame for every group, whereas line sequential scan is performed for every one line for every group. Therefore, line sequential scan is of advantage in minimizing the time lag between wavelength and acquiring a fewer cross-talk image.

NOTE

Image acquisition in the line mode can be performed when you use the FV300 system with AOTF (FV5-COMBA).

2. Select the observation mode with the option buttons in the [Acquire] panel.

3. [Group] (which means each laser) and the <▲> or <▼> buttons appear on the lower part of each [CH] group box.



4. Set the group number according to the reagent in use with the <▲> or <▼> buttons. This setting is not required if the desired number is already displayed.

TIP Image acquisition starts with the smallest group number set.

NOTE **To prevent unwanted color fading, it is recommended to acquire the image from the reagents with longer wavelengths.**

5. Click the <Seq. Once> button to acquire the image.

6. When transmitted observation is also attempted, the [FLUOVIEW] dialog box and the [Lasers] sub-panel appear to specify the laser type which has not been specified yet.

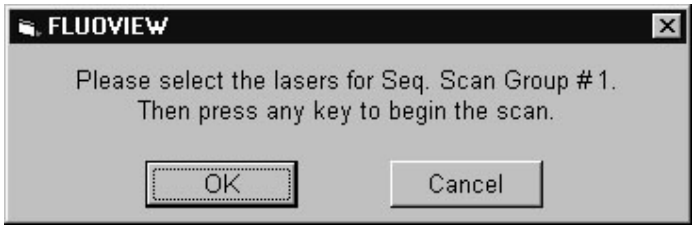


Fig 2-24 [FLUOVIEW] Dialog Box

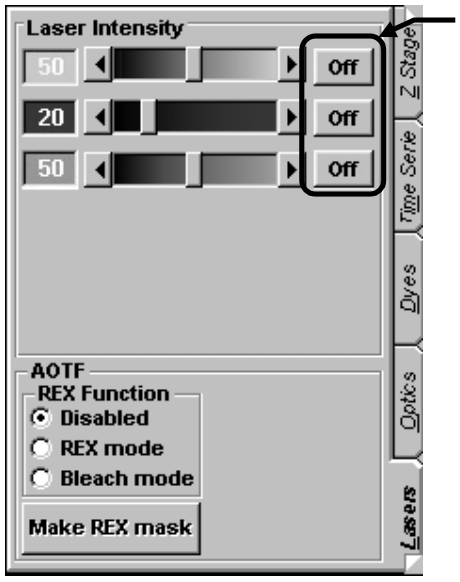


Fig. 2-25 [Lasers] Sub-panel

7. Among the laser <Off> buttons on the right of the [Laser Intensity] scales in the [Lasers] group box, click the <Off> button of the laser you want to use in transmitted observation. This should change the <Off> button to the <Rdy> button (displayed in the pressed-in condition).

8. Click the <OK> button in the [FLUOVIEW] dialog box.
   Image acquisition starts.

9. Adjust the brightness with the [PMT], [Offset] and [Gain] LED sliders in the [Ch] group box in the [Acquire] panel. See section 2-2-1-4-9, “Adjusting the Image Brightness” for details.

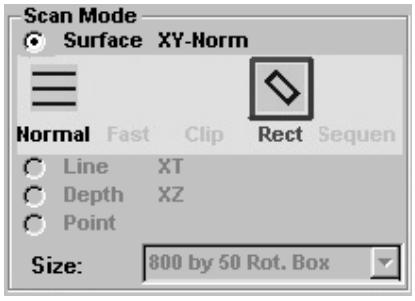
10. After acquiring images, select <Normal> from the list displayed below the [Surface XY-Norm] option button in the [Scan Mode] group box to set the scanning range to the original setting.

2-2-7 Image Acquisition of Rectangular Position at Desired Angle

When the sample is not standing upright, the image of a rectangular position can be acquired by changing the angle.

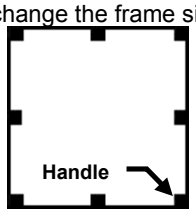
1. Acquire an image in the XY observation mode. See section 2-2-1, “Image Acquisition with XY observation for operation procedure.

2. Select <Rect> under the [Surface XY] option button in the [Scan Mode] group box in the [Acquire] panel. A frame indicating the control range appears in the [Live] panel.

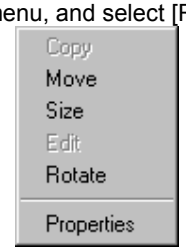


3. Move the frame to the area to be observed. To move the frame, place the mouse pointer inside it and drag the mouse.

4. Change the frame size. Place the mouse pointer inside the frame and click to display square handles around the frame. Then place the mouse pointer on one of these handles and drag it to change the frame size.



5. Change the inclination angle of the frame. Click the mouse right button inside the frame to display the pop-up menu, and select [Rotate] in it.



6. The inclination angle of the frame can be varied according to the mouse pointer movement.
   Place the mouse pointer on an inclining position and click the mouse left button to fix the frame inclination angle.

7. Acquire the image with the buttons such as the <XYZ>, <XYT>, <XYZT>buttons.

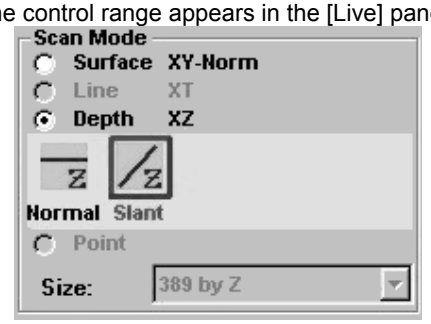
8. After acquiring the image, select <Normal> under the [Surface XY] option button in the [Scan Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-8 Image Acquisition of a Line at Desired Angle

When the sample is not standing upright, the image of a line can be acquired by changing the angle.

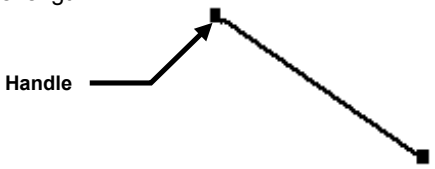
1. Acquire an image in the XY observation mode. For the operation procedure, see section 2-2-1, “Image Acquisition with XY Observation”.

2. Select the [Line XT] option button or <Slant> under the [Depth XZ] option button in the [Scan Mode] group box in the [Acquire] panel.
   A line indicating the control range appears in the [Live] panel.



3. Move the line to the area to be observed. To move the line, place the mouse pointer on it and drag the mouse.

4. Change the line length. Place the mouse pointer on the line and click to display square handles on the two extremities of the line. Then place the mouse pointer on either handle and drag it to change the line length.



5. Change the inclination angle of the line. Right-click the mouse to display the pop-up menu, and select [Rotate] in it.



6. The inclination angle of the line can be varied according to the mouse pointer movement.
   Place the moue pointer on an inclining position and click the mouse left button to fix the line inclination angle.

7. Acquire the image with the buttons such as the <XYZ>, <XYT>, <XYZT> buttons.

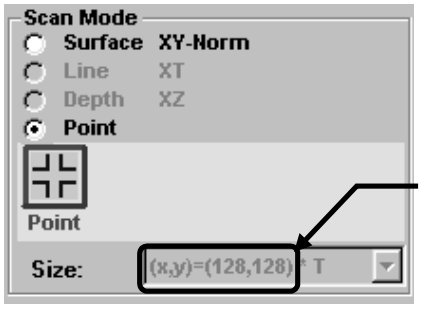
8. After acquiring the image, select <Normal> under the [Surface XY] option button in the [Scan Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-9 Display the change of image intensity (Point Scan)

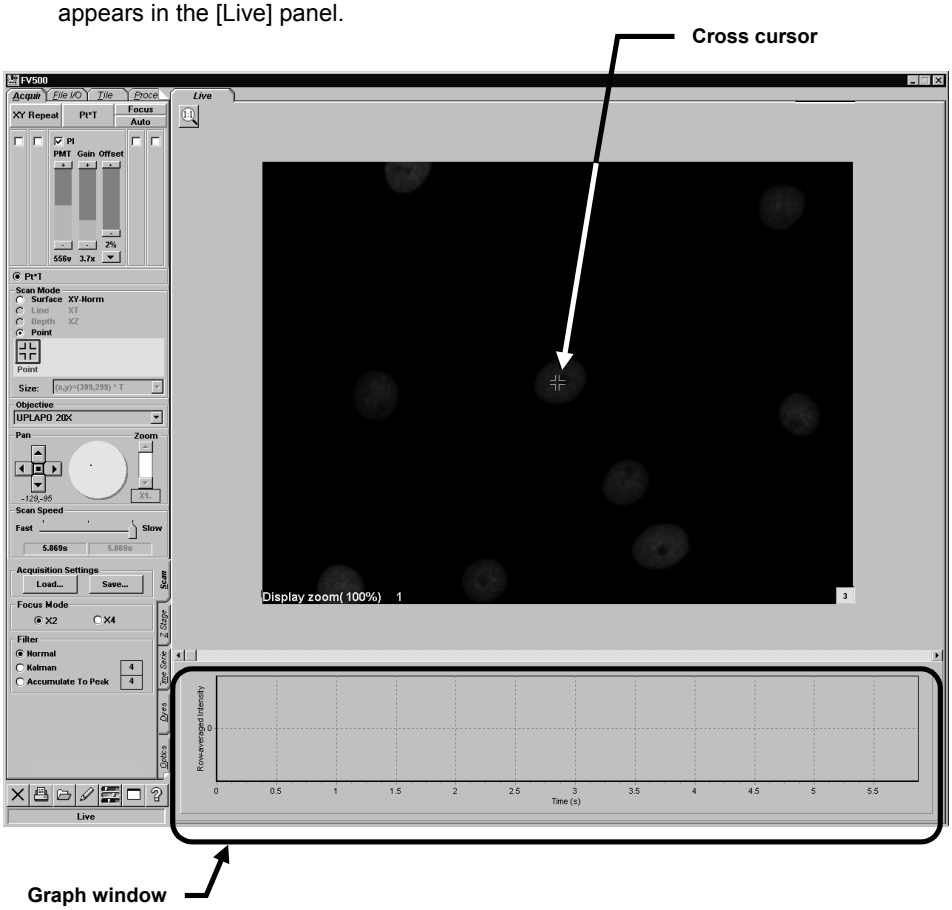
The change of fluorescence intensity according to time-lapse can be displayed graphically by irradiating the laser to certain point on the image.
If you have FLUOVIEW TIME COURSE software, image acquisition can be started with buttons, keys or input of external trigger signals.

1. Acquire an image in the XY observation mode. For the operation procedure, see section 2-2-1, “Image Acquisition with XY observation”.

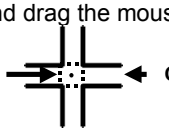
2. Select <Point> under the [Point] option button in the [Scan Mode] group box in the [Acquire] panel.



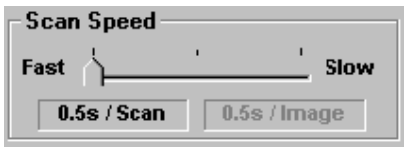
The graph window showing the cross cursor for scanning and the intensity values appears in the [Live] panel.



3. Move the cross cursor to the area you want to observe. To move the cross cursor, place the mouse pointer on it and drag the mouse.

Scan the center of the cross cursor  **Cross cursor**

4. Set the scan speed for image acquisition.
   Set the speed in the [Scan speed] group box of the [Acquire] panel.



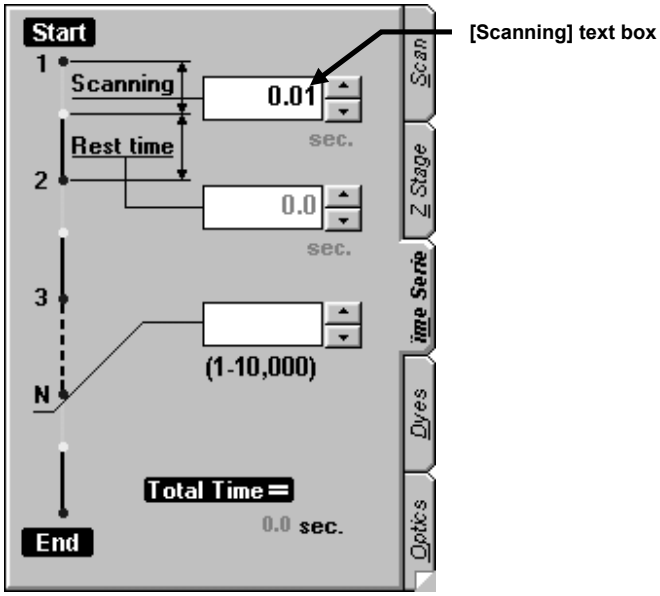
TIP

The speed recommended for point scan are as follows.
2μs(The scroll bar indicates “Fast” position. It is useful for the specimen changes rapidly.), 10μs, 100μs(The scroll bar indicates “Slow” position. It is useful for the specimen changes slowly.)

5. Set the time for measurement.

   From the page tabs on the bottom right of the [Acuire] panel, select the [Time Series] sub-panel.

   Set the time for measurement with the <▲> or <▼> button in the [Scanning] text box.



6. Set the starting method for image acquisition.

   (If you have FLUOVIEW TIME COURSE software)

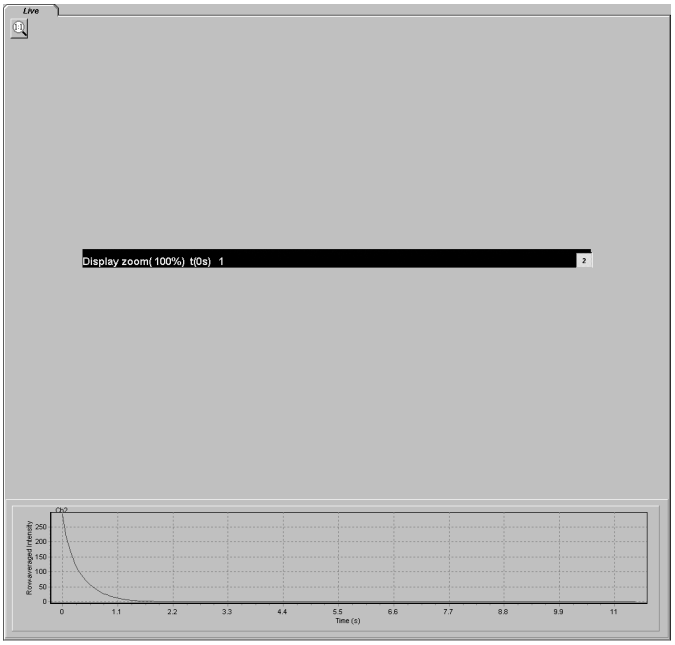
   For setting procedure, see “FLUOVIEW TIME COURSE software user’s manual”.

7. Click the <Pt^{x}T> button to acquire the image.

   The image at the coordinates set in the [Live] panel appears in the image display area and the graph showing the fluorescence intensity also appears in the graph window.

TIP

The image shown in the [Live] panel after image acquisition is the image that acquired at the coordinates specified in the [Live] panel and arranged in the X direction, from the top left to the bottom right.
The image shown after image acquisition is the same a width as that in the X direction specified in the [Live] panel.



TIP

The size of the graph window can be modified by double-clicking the mouse button on it.

TIP

When a certain area is specified by dragging the mouse from the top left to the bottom right on the graph while pressing down its left button, the specified area can be magnified.

TIP

When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP

The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse on the magnified graph from the bottom left to the top right, the top right to the bottom left or the bottom right to the top left.

TIP When click the mouse on the X or Y Axis on the graph window, the [Editing] dialog box appears and the graph parameters or the graph display method can be modified.

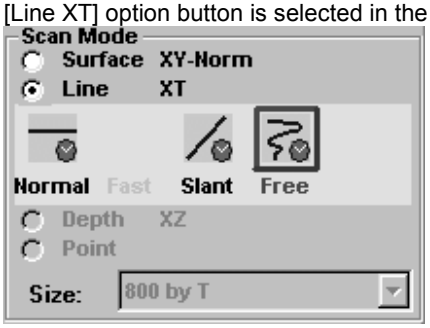
8. After image acquisition, select <Normal> under the [Surface XY] option button in the [Scan Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-10 Image Acquisition on Desired Line (XZ, XT or XZT Observation)

When the sample is in curved form, the image can be acquired by drawing a desired line on it.

1. Acquire an image in the XY observation mode. For detailed operation, see section 2-2-1, “Image Acquisition with XY observation”.

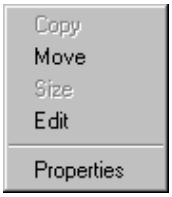
2. Select [Line XT] option button in the [Scan Mode] group box in the [Acquire] panel or <Free> under the [Depth XZ] option button. A line indicating the scanning range appears in the [Live] panel.
   (<Free> under the [Line XT] option button is selected in the following Fig.)



TIP A line can be specified for one display.

3. Move the curved line to the area you want to observe. To move the curve, place the mouse pointer in the box and drag the mouse.

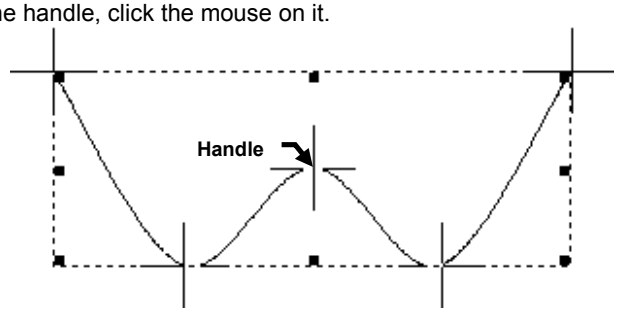
4. Change the curved line shape. Place the mouse pointer within the box and right click to display the pop-up menu. Then select <Edit>.



The cross shaped handles appear around the curve. Place the mouse pointer on either handle and drag it to change the line shape. The color of the mouse pointer is changed on the handle.

The handle can be add by clicking the mouse on desired coordinates.

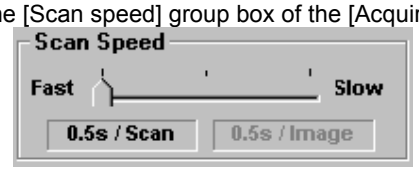
To delete the handle, click the mouse on it.



Right click the mouse on the coordinates but the handles to fix the shape after the change.

5. Set the scan speed for image acquisition.

Set the speed in the [Scan speed] group box of the [Acquire] panel.



TIP

The speed recommended for this scan mode are as follows.

2μs: The scroll bar indicates “Fast” position. It is useful for the specimen changes rapidly.

4μs, 8μs : The scroll bar indicates “Slow” position. It is useful for the specimen changes slowly.

6. Set the interval time or the number of scans.
   The operating procedure is same as that in the XY observation mode. See section 2-2-2, “XT Observation Mode” for details.

TIP

Up to 8000 times, images can be acquired. Set the number of scans in the [N] text box in the [Time Series] sub-panel.

7. Click <XZ>, <XT>, or <XZT> etc. to acquire the image.

TIP

The image shown in the [Live] panel after image acquisition is the image that acquired at the curved line specified on the image and arranged uprightly in the X direction, from the top to bottom.
The image shown after image acquisition is the same a width as that in the X direction specified in the [Live] panel. (Same as that in the XT observation mode).

8. After image acquisition, select <Normal> under the [Surface XY] option button in the [Scan Mode] group box in the [Acquire] panel to return from the image acquisition mode.

2-2-11 Image Acquisition in the Laser Excitation Mode

When you use the FV system with AOTF (FV5-COMBA), the function described in this section is available.

In the FV system with AOTF, it is possible to cut excitation of laser except the region where scanning is performed. Moreover, it is also possible to set up a region of laser excitation to excite laser only to the target part.

TIP

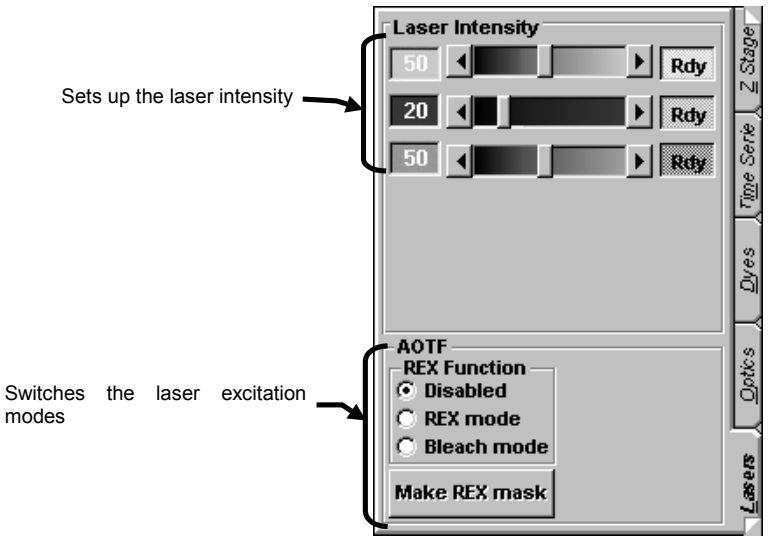
REX represents the “region of laser excitation” in this section.

Three kinds of the laser excitation mode, Disable, REX, and Bleach, are selectable according to your purpose. And each mode can be switched in the XYT observation. In use of the REX or Bleach mode, a setup of REX mask file is required.

Disable mode: An ordinary mode to acquire an image of specified size.

REX mode: The mode which sets the REX mask file to excite laser only to the target part and acquire image. The same laser setup as Disable mode is applied.

Bleach mode: The REX mask file other than that of the REX mode can be set. And each laser setup can also be obtained apart from the Disable/REX mode.

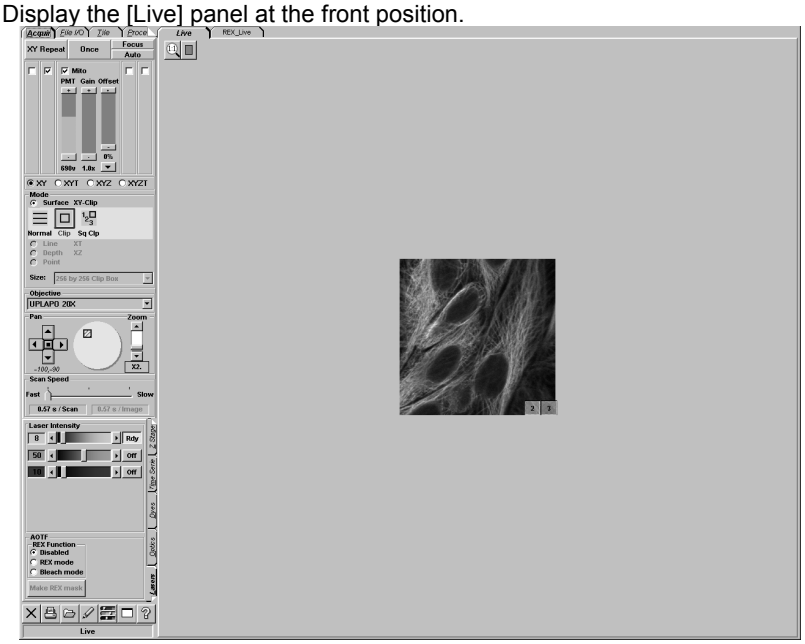


NOTE **In the REX and Bleach mode, image acquisition in the Focus mode, fast scan, and image acquisition in the Line mode (Normal, Slant, and Free) can not be performed.**

NOTE **The specimen in the images carried in this section is not for FRAP, but for the user's manual.**

2-2-11-1 Making REX Mask File

1. Display the [Live] panel at the front position.

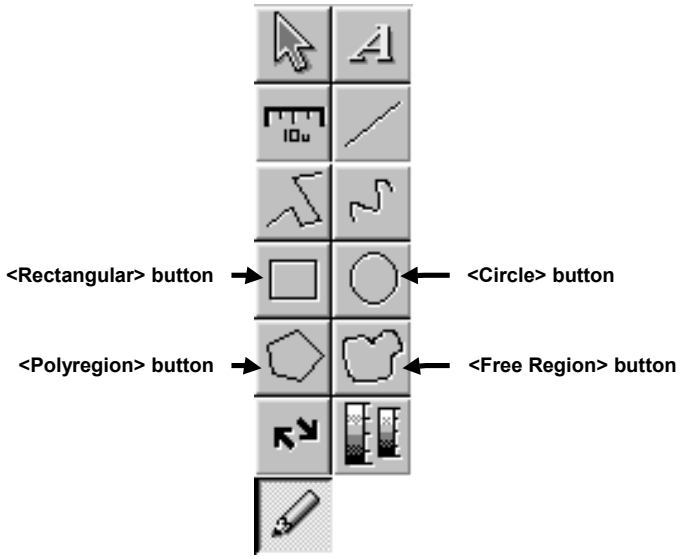




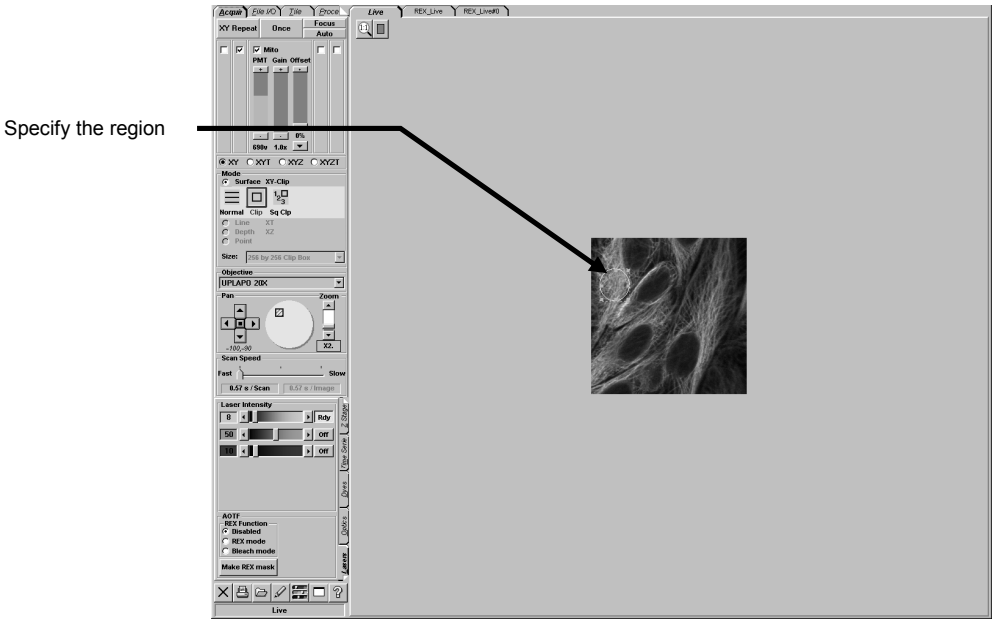
<Annotate> button

2. Select the <Annotate> button in the tool bar at the bottom left of the screen.

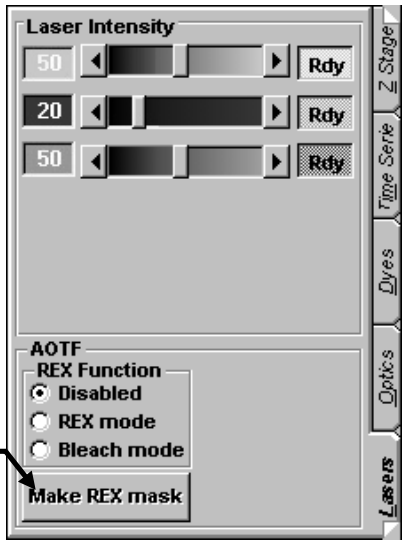
3. In the list of buttons displayed as shown below, select the <Rectangular>, <Circle>, <Polyregion>, or <Free region> button. And drag on the image to specify the region of laser excitation.



TIP In order to specify two or more regions, click the mouse on or inside the region with holding down the SHIFT key after a region is specified.



4. From the page tabs on the bottom right of the [Acquire] panel, select the [Lasers] sub-panel.

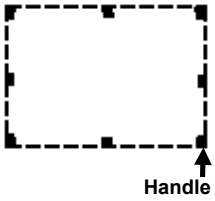


<Make REX mask> button
Makes the REX mask file of the specified region.

Fig. 2-26 [Lasers] sub-panel

TIP

When the <Make REX mask> button is displayed in gray, click and select the specified region on the [Live] panel. When two or more regions are specified, select all the regions to be masked and let the handles be displayed around each region.



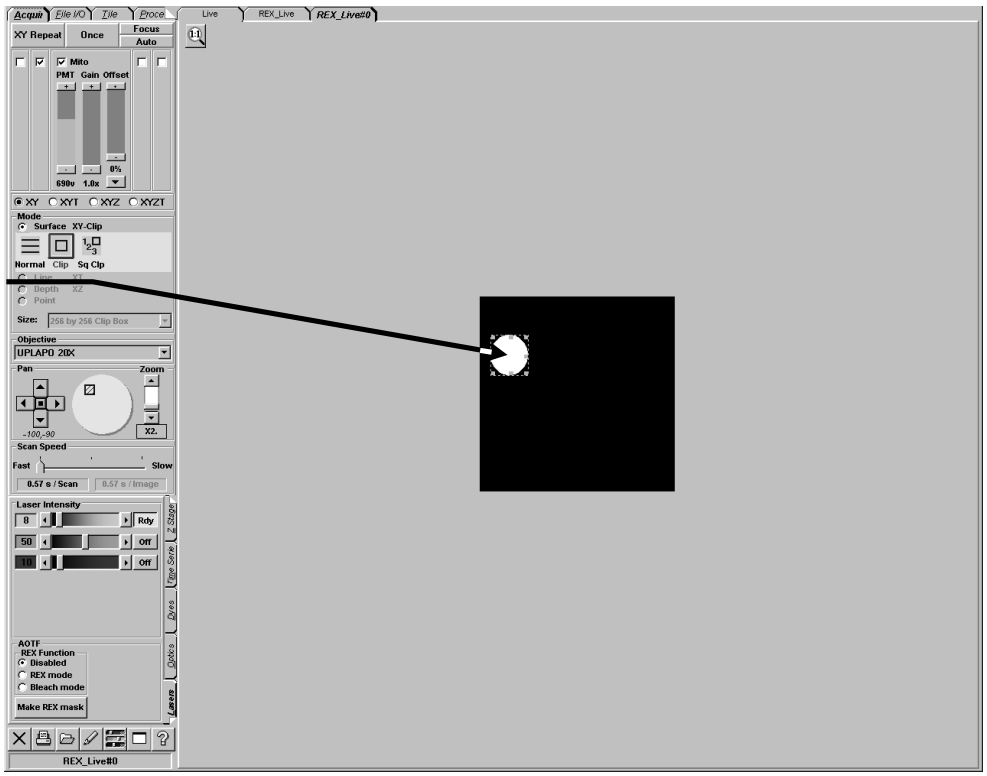


<Make REX mask> button

5. Select the <Make REX mask> button.

   The [REX Live] panel (the REX mask file) is made in the [Display] panel.

Make the REX mask file which masks the region outside of the specified region.



TIP

When the [REX Live] panel has already been created on the FV300 software, A panel is continuously created after [REX_Live0#].

TIP

The REX mask file can be saved.

The saving procedure is completely the same as that of a display.

For details, see section 2-3-1-2, “Saving a Display" in this manual.

6. In order to adjust the laser intensity for every region, right-click the mouse on the white region on the [Display] panel where the REX mask file is displayed. The pop-up menu as shown below appears.

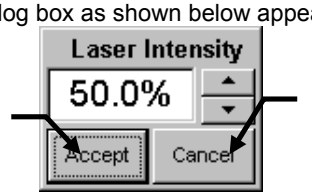


7. Select [Set intensity] in the menu.

   The [Laser Intensity] dialog box as shown below appears.

<Accept> button

Accepts the value set in the [Laser Intensity] text box and closes the dialog box.



<Cancel> button

Cancels the value set in the [Laser Intensity] dialog box and closes the dialog box.

8. Set the value for the laser intensity on percentage and click the <Accept> button.

   The brightness of the region is changed according to the value set in the [Laser Intensity] dialog box.

TIP The laser intensity of the REX mask file is setup on percentage to 100%.

TIP The region becomes brighter as the value is close to “100”.

NOTE **When the laser intensity has been set to other than 100% in the [Laser Intensity] group box in the [Lasers] sub-panel, the value can be decreased in the [Laser Intensity] dialog box. However, the laser intensity value does not equal to the value obtained by multiplying the value in the [Laser Intensity] group box by the percent in the [Laser Intensity] dialog box.**

2-2-11-2 Example of FRAP experiment

The example of procedure of FRAP experiment using AOTF is described in this section.

In order to perform photobleaching to a specimen, the value of laser intensity is raised to 100% to acquire an image since strong laser intensity is temporarily required. When 100% or more of laser intensity value is required, zoom magnification is gathered to acquire an image. (It is because the square power of zoom magnification per time can be secured.) Moreover, using the objective of larger NA concentrates the laser irradiation spot in Z direction, and stronger laser can be irradiated.
Therefore, a preliminary experiment is required to set up the laser intensity, the objective, the zoom ration of the REX mask file according to a specimen.

1 Preliminary Experiment

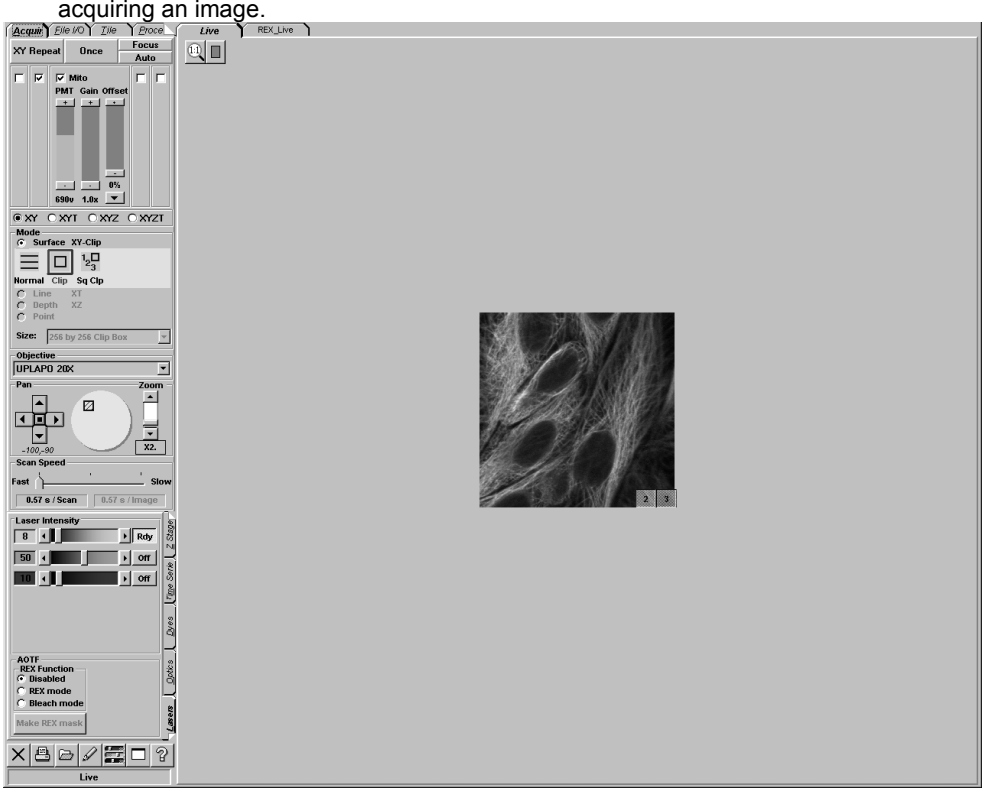
1. Set the laser intensity, the objective, and the zoom ration in the [Acquire] panel so that they are suitable for a specimen.

2 Acquiring Image in XY mode

1. Confirm that [XY-Norm] is displayed in the [Scan Mode] group box in the [Acquire] panel.

2. Select the [XY] option button in the [Acquire] panel.

3. Select the <Once> button in the [Acquire] panel and acquire an image.
   If necessary, set the range for image acquisition with clip scan, for example, after acquiring an image.



3 Making REX Mask File

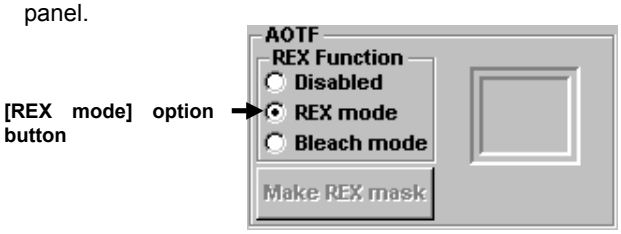
See section 2-2-11-1, “Making REX Mask File” for the procedure to make the REX mask file.

If the setup of the region of laser excitation remains after making the REX mask file, the intensity change of the region can be observed in the TIME COURSE software (optional).
See the “FV-TIEMPO TIME COURSE software User’s manual” for details of the TIME COURSE software.

4 Selecting REX Mask File and Setting the Laser ON/OFF

Set the REX mask file in the REX mode and Bleach mode, and the laser ON/OFF respectively.

1. Select the [REX mode] option button in the [AOTF] group box in the [Lasers] sub-panel.



2. A frame to specify the REX mask file appears on the right side of the [REX mode] option button. Right-click the mouse inside the frame to display the pop-up menu as shown below.

   Select the image to be masked in the menu.



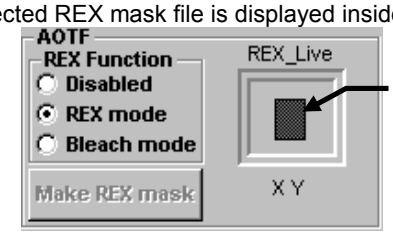
The REX mask file displayed in the [Display] panel is pop-up displayed.

TIP

In order to use the already saved file as a REX mask file, open the image beforehand.

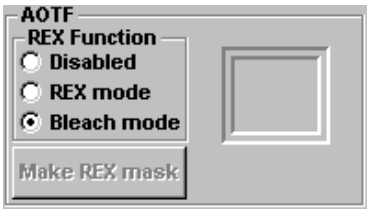
The opening method of the REX mask file is completely the same as that of an image. For details, see section 2-3-2, “Opening Previously Saved Images” in this manual.

3. The icon of the selected REX mask file is displayed inside the frame.



The icon of the selected REX mask file is displayed inside the frame. And the file name and the observation mode are also displayed above and under the frame.

4. Select the [Bleach mode] option button in the [AOTF] group box of the [Laser] sub-panel.



5. A frame to specify the REX mask file appears on the right side of the [Bleach mode] option button. Right-click the mouse inside the frame to display the pop-up menu as shown below.

   Select the image to be masked in the menu.



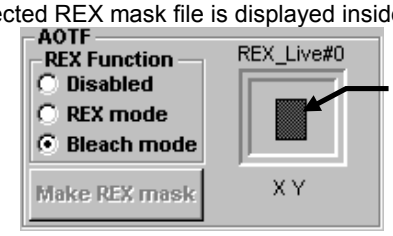
The REX mask file displayed in the [Display] panel is pop-up displayed.

TIP

In order to use the already saved file as a REX mask file, open the image beforehand.

The opening method of the REX mask file is completely the same as that of an image. For details, see section 2-3-2, “Opening Previously Saved Images” in this manual.

6. The icon of the selected REX mask file is displayed inside the frame.



The icon of the selected REX mask file is displayed inside the frame. And the file name and the observation mode are also displayed above and under the frame.

7. Set each laser ON/OFF, and their intensity.

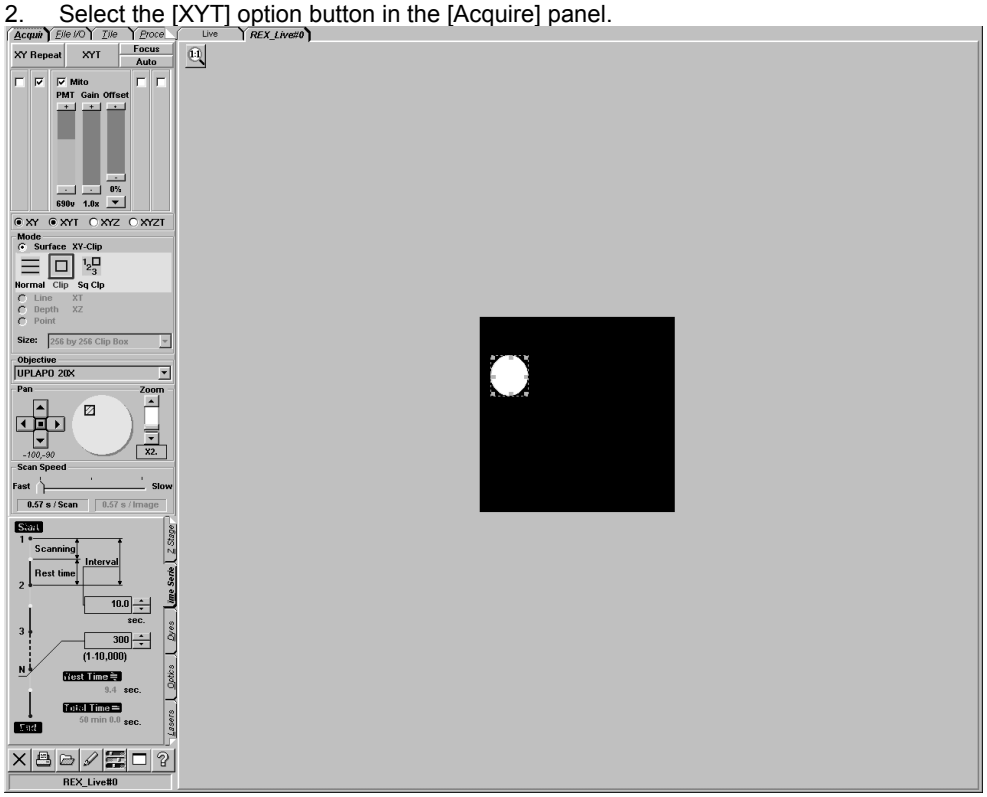
   If necessary, use the [Laser Intensity] dialog box in the [Lasers] sub-panel to set the laser ON/OFF.

   And the value set in the [Laser Intensity] dialog box can be obtained apart from the value set in the Disable/REX mode.

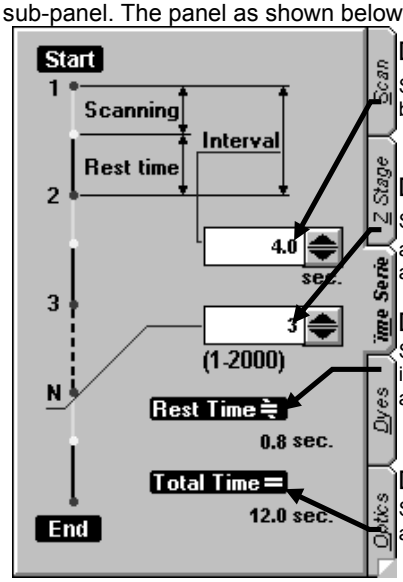
5 Setting the XYT Observation mode

1. Confirm that [XY-Norm] is displayed in the [Scan Mode] group box in the [Acquire] panel.

2. Select the [XYT] option button in the [Acquire] panel.



3. From the page tabs on the bottom right of the [Acquire] panel, select the [Time Series] sub-panel. The panel as shown below appears.



[Interval] text box
Set the interval with the < ▲ > and< ▼ > buttons. Key entry is also acceptable.

[N] text box
Set the number of scans with the < ▲ > and < ▼ > buttons. Key entry is also acceptable.

[Rest Time] text box
Shows the time after the end of an image acquisition until the start of next acquisition

[Total Time] text box
Shows the time required to complete image acquisition.

Fig. 2-27 [Time Series] sub-panel

4. Set the interval time to “10” using the < ▲ > and < ▼ > buttons in the [Interval] text box. Change the interval time corresponding to the specimen.

5. Set the number of scans to “30” using the < ▲ > and < ▼ > buttons in the [N] text box in the [Time Series] sub-panel. Change the number of scans corresponding to the specimen.

6. Confirm that the time for image acquisition is set to 5 minutes (or 300 seconds) in the [Total Time] text box. The time for image acquisition differs according to the interval and the number of scans.

6 Setting the TIME COURSE Software (optional)

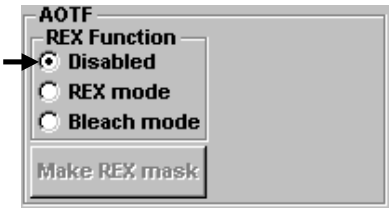
Setting of the real time graph is required when you use the TIME COURSE software. See the “FV-TIEMPO TIME COURSE software User’s manual” for details of the TIME COURSE software.

7 Acquiring Data

The experiment data before and after photobleaching of certain region can be obtained after performing an ordinary image acquisition in the first scan, an image acquisition in the Bleach mode in the second scan , and an ordinary image acquisition in the third scan. When the REX mode is selected in the third scan, the data of the region other than the specified region can not be obtained since the laser is irradiated only to the specified region. However, the REX mode is effective to prevent fading of the obtained data.

1. Select the [Disabled] option button in the [AOTF] group box in the [Lasers] sub-panel.

[Disabled] option button



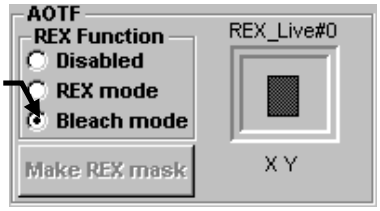


<XYT> button

2. Click the <XYT> button to start image acquisition.

3. Select the [Bleach mode] option button in the [AOTF] group box in the [Lasers] sub-panel.

[Bleach mode] option button

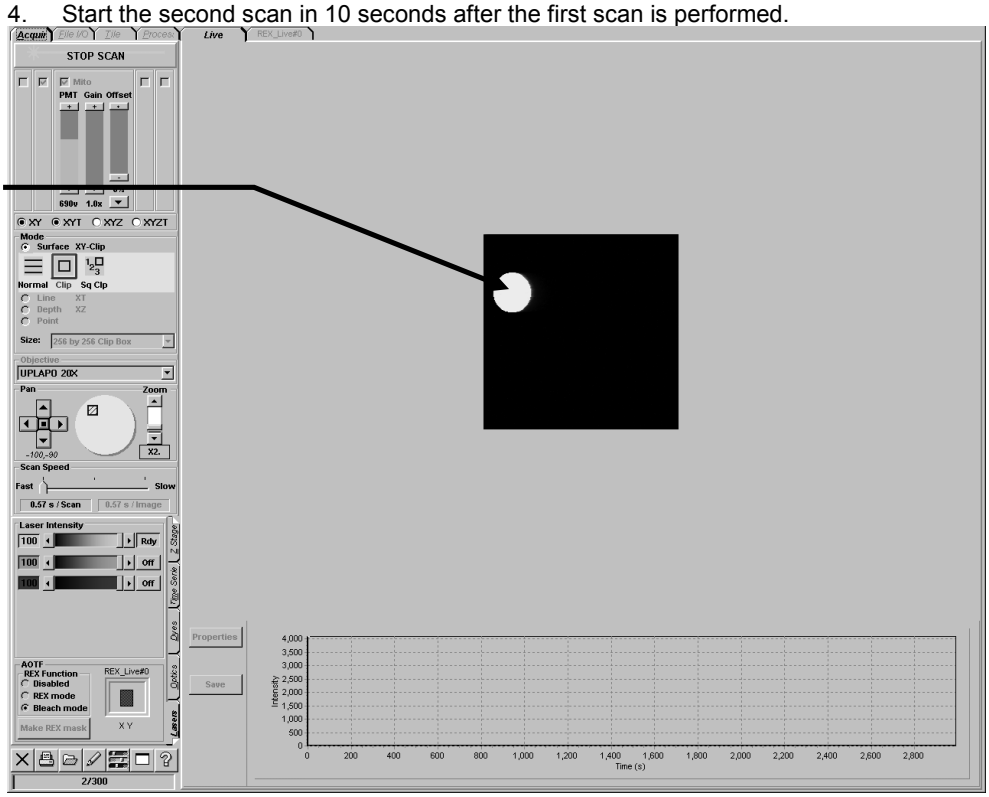


NOTE **Select the [Bleach mode] option button within 10 seconds before the next scanning is started.**

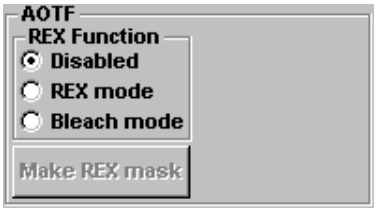
NOTE **In the TIME COURSE software (optional), when the REX mask file in the REX or Bleach mode is changed while image scanning is performed obtaining the real time graph, the laser is irradiated while the pop-up menu is displayed. however, the real time graph is not plotted. Please be aware this before using.**

4. Start the second scan in 10 seconds after the first scan is performed.

The laser of strong intensity is irradiated to the specified region.



5. Select the [Disabled] or [REX mode] option button in the [AOTF] group box in the [Lasers] sub-panel.



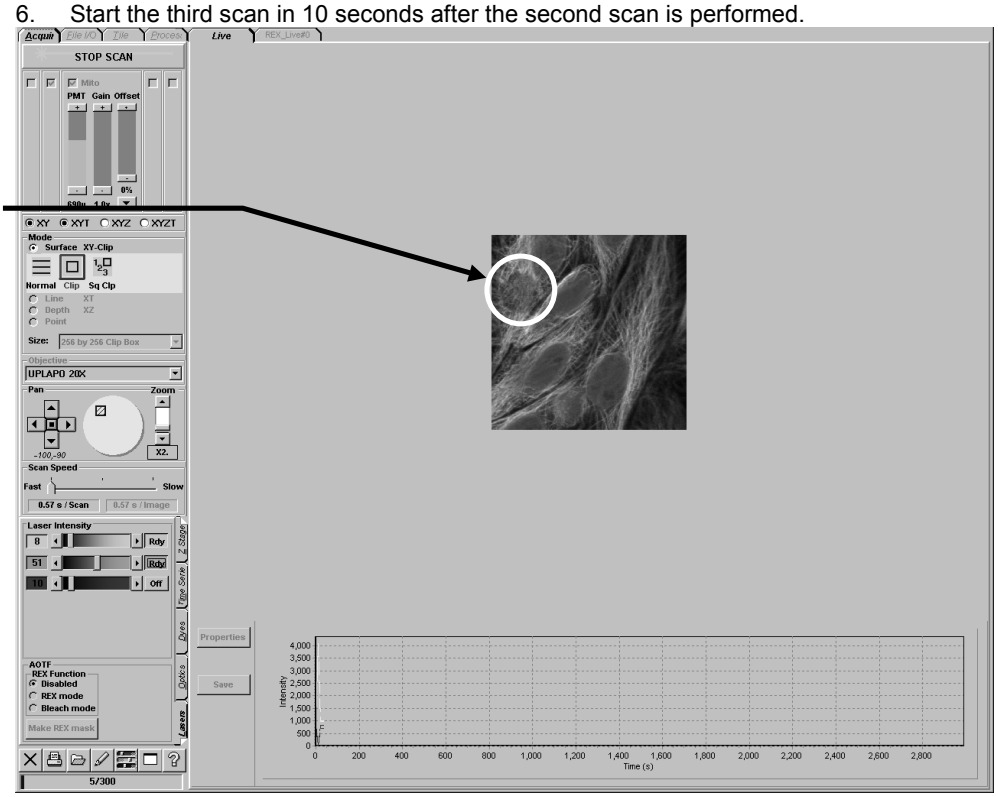
(The [Disabled] option button is selected.)

NOTE **Select the the [Disabled] or [REX mode] option button within 10 seconds before the next scanning is started.**

NOTE **In the TIME COURSE software (optional), when the REX mask file in the REX or Bleach mode is changed while image scanning is performed obtaining the real time graph, the laser is irradiated while the pop-up menu is displayed, however, the real time graph is not plotted. Please be aware this before using.**

6. Start the third scan in 10 seconds after the second scan is performed.

The image of the specimen is fading only where the laser is irradiated.



(The [Disabled] option button is selected.)

7. Start the forth and subsequent scan in 10 seconds after the third scan is performed.

   The image of the photobleached specimen is acquired.

NOTE **When performing the forth or subsequent scan, be sure to switch the [REX mode] and [Bleach mode] between scans.**

NOTE **In the TIME COURSE software (optional), when the REX mask file in the REX or Bleach mode is changed while image scanning is performed obtaining the real time graph, the laser is irradiated while the pop-up menu is displayed, however, the real time graph is not plotted. Please be aware this before using.**

2-3 Saving, Opening and Shredding Images

Use the [File I/O] panel to save, open or shred an image.

Display the [File I/O] panel at the front.

[Open]

To open a file, select the desired file in the [Files] list box and drag the file here.

[File Type] drop-down list

Selects the type of the files to be displayed in the [Files] list box.

[Files] list box

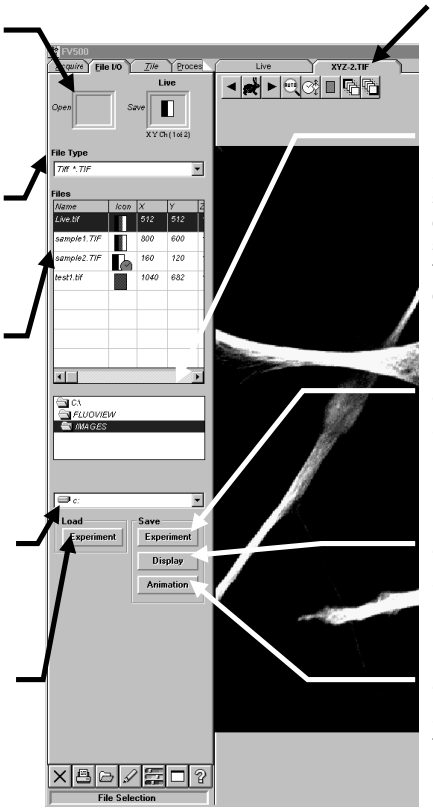
Lists the image files of the type selected with the [File Type] drop-down list and their acquisition parameters. Select the file to be opened from this list. The files in the list can be viewed by scrolling.

[Drive] drop-down list

Click < ▼ > to display the existing drives and select one of them.

<Experiment> button

Click to open the selected image file.



[Display] panel

Displays the image. The image file name is shown in the panel page tab.

[Directory] list box

Shows the directories in the hierarchical structure. Double-click the required directory. If the required directory is not shown, double-click the upper level of the current directory or the root directory (\ ).

<Experiment> button

Saves the image displayed in the [Display] panel together with its file name.

< Display> button

Saves the image displayed in the [Display panel] as if a hardcopyed image.

<Animation> button

Saves the animation image displayed in the [Display panel].

Fig. 2-28 [File I/O] Panel

<Image Icons>

Images are represented by icons which can also identify the observation modes used when acquiring them.

The icon of the selected image (image in the [Display] panel) is displayed in the frame at the top of the function panel such as the [File I/O] panel.

In the [File I/O] panel, image icons are also displayed in the [Icon] field in the [Files] list box.

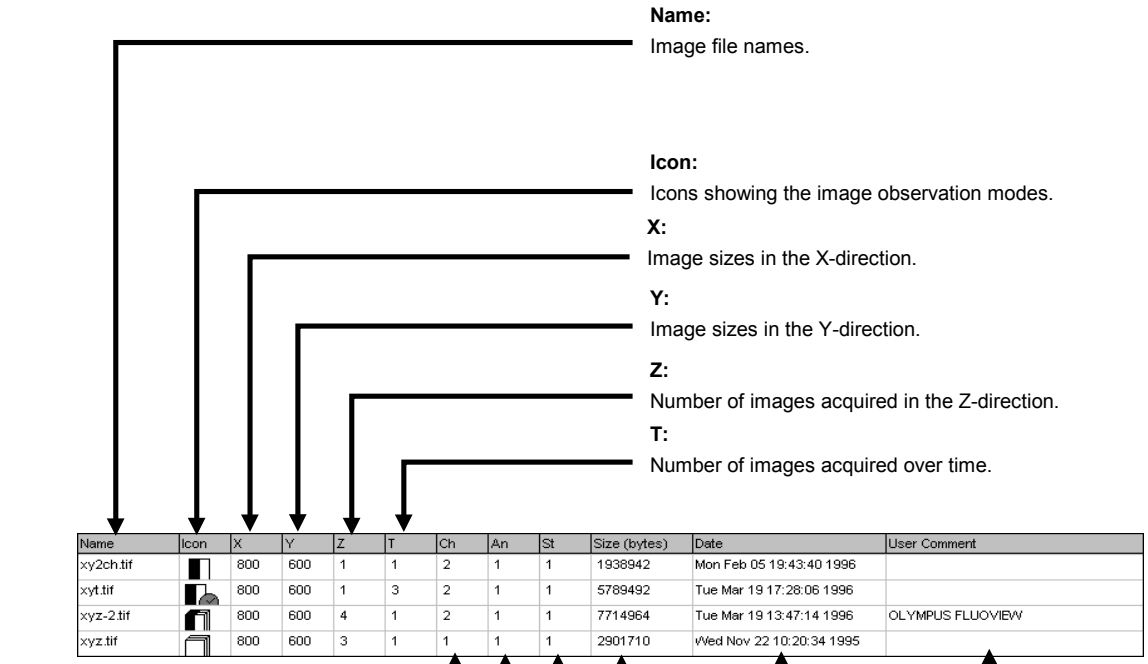
TIP In all observation modes, the icons for 3 or more channels are identical.

Image Icon	Significance
	XZ observation
	XZ observation, 2-channel mode
	Xt observation
	Xt observation, 2-channel mode
	XZT observation
	XZT observation, 2-channel mode
	XY observation
	XY observation, 2-channel mode
	XYt observation
	XYt observation, 2-channel mode
	XYZ observation
	XYZ observation, 2-channel mode
	XYZt observation
	XYZt observation, 2-channel mode
	Point Scan
	Animation image
	Stereo 3D image: Image to be viewed with color eyeglasses.
	3 or more channels

<[Files] List Box>

The enter view of this list box cannot be displayed.

Scroll the entries when viewing this list box.



Name	Icon	X	Y	Z	T	Ch	An	St	Size (bytes)	Date	User Comment
xy2ch.tif		800	600	1	1	2	1	1	1938942	Mon Feb 05 19:43:40 1996	
xyt.tif		800	600	1	3	2	1	1	5789492	Tue Mar 19 17:28:06 1996	
xyz-2.tif		800	600	4	1	2	1	1	7714964	Tue Mar 19 13:47:14 1996	OLYMPUS FLUOVIEW
xyz.tif		800	600	3	1	1	1	1	2901710	Wed Nov 22 10:20:34 1995	

Name:
Image file names.

Icon:
Icons showing the image observation modes.

X:
Image sizes in the X-direction.

Y:
Image sizes in the Y-direction.

Z:
Number of images acquired in the Z-direction.

T:
Number of images acquired over time.

Ch:
Number of image channels.

An:
Number of images created when constructing a stereo image using the Visualize function.

Stx
"2" is displayed when stereo images (stereo 3D image, or stereo image to be viewed through color eyeglasses) are constructed and saved using the Visualize function.

Size:
Image file sizes.

Date:
Image saving dates.

User Comment:
Part of the comment saved with each image.

2-3-1 Saving Images

Either a series of images or single image being displayed can be saved.

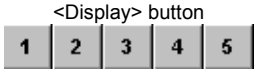
2-3-1-1 Saving Images As a Series

The displayed images can be saved in a disk as a series image file.


<Set start position> button


<Set stop position> button


<Display> button



<Display channel switch> buttons

1. Display the [Display] panel showing the image to be saved at the front.
2. When there is more than one image to be saved, the <Set start position> and <Set end position> buttons will be displayed on the upper part of the image. Display the image with which the saving should start using the <Display> buttons, then click the <Set start position> button.
   Also set the save end position in the similar way.
3. When the images to be saved are acquired from more than one channel, select whether saving images from more than one channel simultaneously or only a single image from a single channel.
   Use the <Display channel switch> buttons for this selection. The images will be saved according to the conditions of the selected channels.
   Example) When only the image of Channel 1 is displayed, only the image of Channel 1 will be saved.

TIP

For the channel switching, see section 2-4-3, “Switching the Displayed Channels”.

4. Click the <Experiment> button in the [Save] group box. The [Save Experiment As] dialog box as shown below appears.

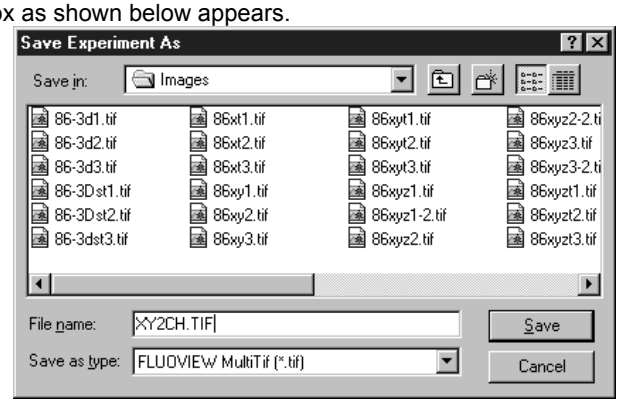


Fig. 2-29 [Save Experiment As] Dialog Box

5. When it is required to change the save destination drive or directory, use the [Save in:] drop-down list.
6. When it is required to change the saved file type, use the [Save as Type:] drop-down list. See section 2-3-1-5, “File Types Available for Save” for details.
7. Enter the file name in the [File Name:] text box.
8. Click the <Save> button.

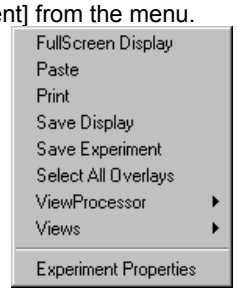
NOTE

If a file with the same name as the entered file name already exists, a dialog box is displayed to ask if you want to overwrite the existing file. If you do not want to overwrite it, click the <NO> button and enter another file name.

One Point!

The [Save Experiment As] dialog box can also be displayed by a mouse operation.

1. Display the image to be saved at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [Save Experiment] from the menu.



2-3-1-2 Saving a Display

The displayed images can be hardcopied and saved in a disk. This method is used when it is required to use a FLUOVIEW image in another application.


<Display> button

1. Display the [Display] panel showing the image to be saved at the front.
2. When there is more than one image to be saved, display the image to be saved using the <Display> buttons.
3. Click the <Display> button in the [Save] group box. The [Save Display] dialog box as shown below appears.

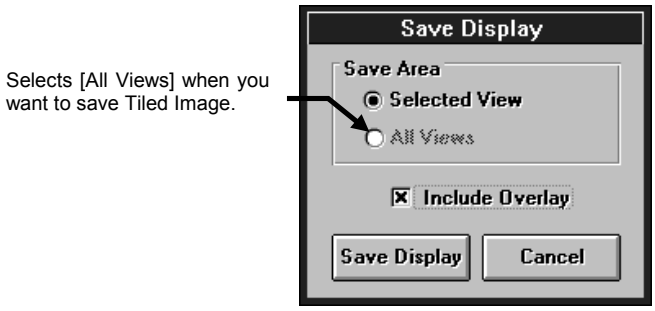


Fig. 2-30 [Save Display] Dialog Box

4. When it is required to save the comment drawn on the image together with the image, check the [Include Overlay] check box.
5. Click the <Display> button in the [Save] group box. The [Save Experiment As] dialog box as shown below appears.

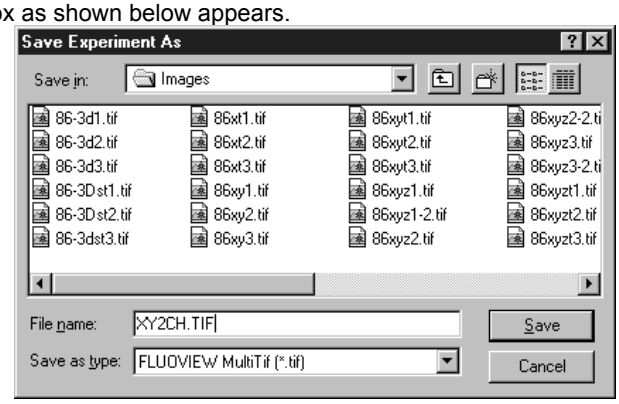


Fig. 2-31 [Save Experiment As] Dialog Box

6. When it is required to change the save destination drive or directory, use the [Save in:] drop-down list.

7. When it is required to change the saved file type, use the [Save as Type:] drop-down list. See section 2-3-1-4, “File Types Available for Save” for details.

8. Enter the file name in the [File Name:] text box.

9. Click the <Save> button.

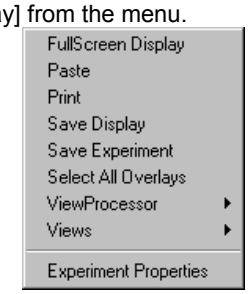
NOTE

If a file with the same name as the entered file name already exists, a dialog box is displayed to ask if you want to overwrite the existing file. If you do not want to overwrite it, click the <NO> button and enter another file name.

One Point!

The [Save Display] dialog box can also be displayed by a mouse operation.

1. Display the image to be saved at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [Save Display] from the menu.



2-3-1-3 Saving Specified Area of Image

Only the specified part of an image can be saved as an image in a disk.

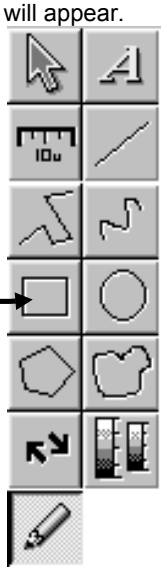
1. Display the [Display] panel of the image containing the part to be saved in the front.
2. When the image was acquired through multiple channels, you can select whether the image slices for multiple channels are saved together or the image slice of only 1 channel is saved.
   Use the <display switching> button for the selection. The image will be saved in the channel condition as displayed.

TIP

For the channel switching, see section 2-4-3, “Switching the Displayed Channels”.

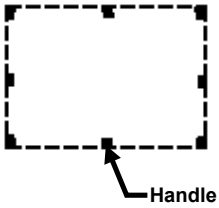

<Annotate> button

3. Specify a desired area in the image. This operation is not necessary if an area has already been specified.
   Click the <Annotate> button in the toolbar at the bottom of the [Acquire] panel. A list of buttons as shown below will appear.



4. Click the <Rectangular> button in this displayed buttons.

5. Specify the area to be saved in the image in the [Display] panel.
   The area is displayed on the image with the handles on its frame.
   The area becomes the save target while these handles are displayed.



6. Click the <Annotate> button so that the list of buttons disappear.

7. Click the <Experiment> button in the [Save] group box.
   The [Save Experiment As] dialog box appears.

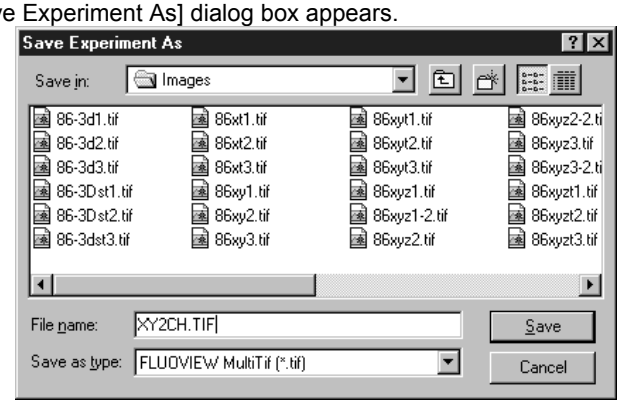


Fig. 2-32 [Save Experiment As] Dialog Box

8. Use the [Save In:] drop-down list if you want to change the save destination drive and directory.

9. Select “FLUOVIEW Multi Tiff” in the [Save as Type:] drop-down list.

TIP

It is not possible to save only the specified area of image in the Single TIF, BMP or microVoxel format.

10. Enter the file name in the [File Name:] text box.

11. Click the <Save> button.

12. A dialog box as shown below appears.

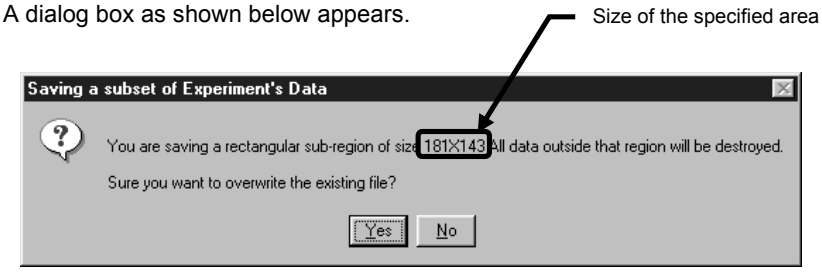


Fig. 2-33 [Saving a subset of Experiment's Data] Dialog Box

TIP

The [Saving a subset of Experiment's Data] dialog box does not appear if no area is specified in the image on the [Display] panel.

13. Click the <Yes> button.

2-3-1-4 Saving Animation Images

Transform a created animation image into the AVI file format and save it in the file type which can display the animation image without this software.

1. Create an animation image.
   Refer to “2-8-2 Animation” for details.
2. Display the [Display] panel of the created image at the front.

TIP

The AVI file is created to save the animation at the display speed mode selected here. For varying the animation display speed, see section 2-8-1-1, “Changing the Successive Display Speed”.

3. Select whether saving images from multiple channels simultaneously or only an image from a channel if the images to be saved are acquired from multiple channels.
   Use the <Display Channel Switch> button for this selection. The image is saved according to the condition of the selected channel.

TIP

For the channel switching, see section 2-4-3 “Switching the Displayed Channels”.



<Save Animation>button

4. Click the <Animation> button in the [Save] group box. The [Save Experiment As] dialog box appears as shown below.

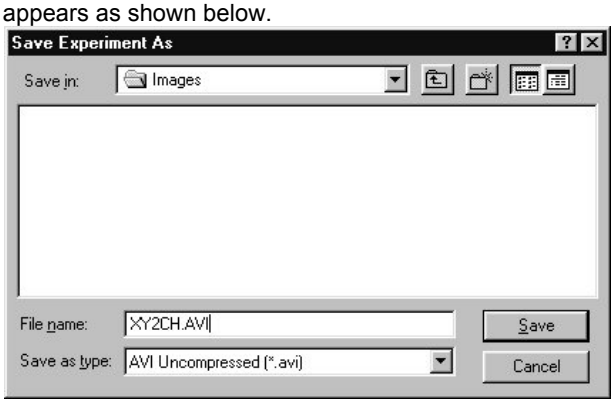


Fig 2-34 [Save Experiment As] Dialog Box

5. When it is required to change the save destination drive or directory, use the [Save in :] drop-down list.
6. When it is required to change the saved file type, use the [Save as Type :] drop-down list.

TIP

Two saving methods can be selected.
AVI compressed : Saves the AVI file being compressed.
AVI Uncompressed : Saves the AVI file not being compressed.

7. Enter the file name in the [File Name:] text box.
8. Click the <Save> button.

NOTE

If a file with the same name as the entered file name already exists, the dialog box appears to ask if you want to overwrite the existing file. If you do not want to do so, click the <No> button and enter another file name.

2-3-1-5 File Types Available for Save

The file type used for saving image in a file can be selected by the user. See section 2-3-1-1, “Saving Images As a Series” for the operation method.

1. Click the <Experiment> button in the [Save] group box.
2. Select the file type from the [Save as Type:] drop-down list in the [Save Experiment As] dialog box. Three file types are available as detailed below.
   - Fluoview Multi Tiff(*.tif): TIFF format designed for use with FLUOVIEW. Used for image analysis, processing, etc., on FLUOVIEW.
   - Single TIF(s) 8-bit(*.tif):
   - Single TIF(s) 24-bit(*.tif): TIFF (Tagged-Image File Format) is used for image exchange between applications or computers. (Two types including the 8-bit and 24-bit types are available.)
   - Bitmap 8-bit(*.bmp):
   - Bitmap 24-bit(*.bmp): The BMP format is the standard raster format of MS-Windows. (Two types including the 8-bit and 24-bit types are available.)
   - mVx Files (*.img,*.ani): File format for microVoxel. Used for exchanging images with microVoxel.

TIP In the file types above, characters inside ( ) indicate the extension when a file is saved.

NOTE **When saving a display, the file format can be selected from Single TIF(s) 8-bit *.tif), Single TIF(s) 24-bit (*.tif), Bitmap 8-bit (*.bmp) and Bitmap 24-bit (*.bmp).**

NOTE **When saving an image obtained by merging more than one channel, use a 24-bit file type.**

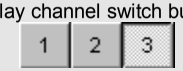
NOTE **If you want to save the image with the comment drawn on it as an image, select the file type according to the usage of the image as described below.**

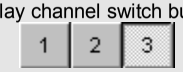
Fluoview Multi Tiff: Select when the image will be analyzed or processed after save. Opening the image also opens the comment, which can be changed or measured as required.

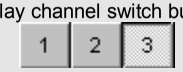
24-bit, 8bit file type: Select when the image will be handed to another application.

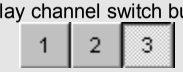
The following table shows the list of image save status by taking the below-mentioned acquisition parameters as an example.

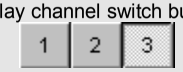
Observation mode:	XYZ observation
Number of channels:	3 channels
Number of acquired images:	4 slices
Save file name:	abcdefgh

	Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving side-by-side images Side-by-side buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Fluoview Multi Tiff	The image of only Ch3 is saved. Save file name: abcdefgh.tif (Ch3: Nos. 0 to 3)	The saved image contains the image slices of the channels used in the above views. The images of Ch1, Ch2 and Ch3 are saved. Save file name: abcdefgh.tif (Ch1·Ch2·Ch3;0-3)	The images of Ch1, Ch2 and Ch3 are saved. Save file name: abcdefgh.tif (Ch1, Ch2, Ch3: Nos. 0 to 3)	The image is saved in the selected channel condition. Note that a file does not always contain the corresponding number of channels. (See “Saving a channel under displayed condition”, “Side-by-side images” and “Merged images” on the left.) The image is saved in the original, non-extended status.	Can be saved.

		Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving side-by-side images Side-by-side buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Single TIF(s)	8-bit	4 images are saved only from Ch3. Save file names: abcdefgh0.tif | (Ch3: No. 0) abcdefgh3.tif (Ch3: No. 3)	The saved image contains the image slices of the channels used in the above views. 4 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefghV0.tif | (Ch1: No. 0) abcdefghV2.tif (Ch3: No. 0) abcdefghV3.tif | (Ch1: No. 1) abcdefghV5.tif (Ch3: No. 1) abcdefghV6.tif | (Ch1: No. 2) abcdefghV8.tif (Ch3: No. 2) abcdefghV9.tif | (Ch1: No.3) abcdefghV11.tif (Ch3: No.3)	4 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Display gradation of the images are poor than original. Save file names: abcdefgh0.tif | (Ch1+Ch2+Ch3: No. 0) abcdefgh3.tif (Ch1+Ch2+Ch3: No. 3)	The image is saved in the selected channel condition. Note that a file does not always contain the corresponding number of channels. (See “Saving a channel under displayed condition”, “Side-by-side images” and “Merged images” on the left.) The image is saved in the original, non-extended status.	Can be saved.

		Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving side-by-side images Side-by-side buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Can be saved.	24-bit	4 images are saved only from Ch3. Save file names: abcdefgh0.tif | (Ch3: No. 0) abcdefgh3.tif (Ch3: No. 3)	4 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefghV0.tif | (Ch1: No. 0) abcdefghV2.tif (Ch3: No. 0) abcdefghV3.tif | (Ch1: No. 1) abcdefghV5.tif (Ch3: No. 1) abcdefghV6.tif | (Ch1: No. 2) abcdefghV8.tif (Ch3: No. 2) abcdefghV9.tif | (Ch1: No. 3) abcdefghV11.tif (Ch3: No. 3)	4 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.tif | (Ch1 + Ch2 + Ch3: No. 0) abcdefgh3.tif (Ch1 + Ch2 + Ch3: No. 3)	The relationship between the saved channels and files are variable depending on the condition of the displayed channels. (See “Saving a channel under displayed condition”, “Side-by-side images” and “Merged images” on the left.) File No. 0 stores image No. 0, file No. 1 stores the image obtained y accumulating images Nos. 0 and 1,..... The image in the last file is the image obtained by accumulating all of the images. Save file names: abcdefg0;tif | (Image No. 0) abcdefg3;tif (Image Nos. 0 + 1 + 2 + 3)	Can be saved.

		Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving side-by-side images Side-by-side buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Bitmap	8-bit	4 images are saved only from Ch3. Save file names: abcdefgh0.bmp | (Ch3: No. 0) abcdefgh3.bmp (Ch3: No. 3)	The saved image contains the image slices of the channels used in the above views. 4 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefghV0.tif | (Ch1: No. 0) abcdefghV2.tif (Ch3: No. 0) abcdefghV3.tif | (Ch1: No. 1) abcdefghV5.tif (Ch3: No. 1) abcdefghV6.tif | (Ch1: No. 2) abcdefghV8.tif (Ch3: No. 2) abcdefghV9.tif | (Ch1: No.3) abcdefghV11.tif (Ch3: No.3)	4 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Display gradation of the images are poor than original. Save file names: abcdefgh0.tif | (Ch1+Ch2+Ch3: No. 0) abcdefgh3.tif (Ch1+Ch2+Ch3: No. 3)	The image is saved in the selected channel condition. Note that a file does not always contain the corresponding number of channels. (See “Saving a channel under displayed condition”, “Side-by-side images” and “Merged images” on the left.) The image is saved in the original, non-extended status.	Can be saved.

		Saving a channel under displayed condition (Example: When only Ch3 is displayed) Display channel switch buttons 	Saving side-by-side images Side-by-side buttons 	Saving merged images (Example: When Ch1, Ch2 and Ch3 are displayed) Display channel switch buttons 	Saving extended image Z/T series switch button 	Comment drawn on images
Bitmap	24-bit	4 images are saved only from Ch3. Save file names: abcdefgh0.bmp | (Ch3: No. 0) abcdefgh3.bmp (Ch3: No. 3)	4 images are saved from each of Ch1, Ch2 and Ch3. Save file names: abcdefghV0.tif | (Ch1: No. 0) abcdefghV2.tif (Ch3: No. 0) abcdefghV3.tif | (Ch1: No. 1) abcdefghV5.tif (Ch3: No. 1) abcdefghV6.tif | (Ch1: No. 2) abcdefghV8.tif (Ch3: No. 2) abcdefghV9.tif | (Ch1: No.3) abcdefghV11.tif (Ch3: No.3)	4 images obtained by merging Ch1, Ch2 and Ch3 images are saved. Save file names: abcdefgh0.bmp | (Ch1 + Ch2 + Ch3: No. 0) abcdefgh3.bmp | (Ch1 + Ch2 + Ch3: No. 3)	The relationship between the saved channels and files are variable depending on the condition of the displayed channels. (See “Saving a channel under displayed condition”, “Side-by-side images” and “Merged images” on the left.) File No. 0 stores image No. 0, file No. 1 stores the image obtained y accumulating images Nos. 0 and 1,..... The image in the last file is the image obtained by accumulating all of the images. Save file names: abcdefgh0;bmp | (Image No. 0) abcdefgh3;bmp (Image Nos. 0 + 1 + 2 + 3)	Can be saved.

2-3-2 Opening Previously Saved Images

Image files saved in the disk can be opened as follows.

1. If the image file name that you want to open is not displayed in the [Files] list box, change the drive and/or directory to those containing the desired file using the [Drive] drop-down list and/or [Directory] list box.
2. From the [File Type] drop-down list, select the file type of the files to be listed in the [Files] list box.
3. Click the <Experiment> button in the [Load] group box.

TIP

Other methods are also available for opening a file:

Perform the same operations as steps 1 and 2 above before the following.

- Place the mouse pointer on the desired image file name in the [Files] list box and drag the file name to the [Open] frame on the upper part of the [File I/O] panel.
  (The mouse pointer transforms to an image icon during dragging.)
- Place the mouse pointer on the desired image file name in the [Files] list box and double-click it.

2-3-3 Shredding Images

Shredding an image refers to removing it from the objects of processing including display. **Shredding does not actually deletes the image saved in the disk**.



<Experiment List> button

1. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.



Fig. 2-35 [Experiments in Memory] Dialog Box


<Shredder> button

2. In the [Experiments in Memory] dialog box, select the file name of the image to be shredded and click the <Shredder> button.
   The file can also be shredded by placing the mouse pointer on it and dragging it to the <Shredder> button.

TIP

The mouse pointer transforms to an image icon during dragging.

3. Click the <Done> button in the [Experiments in Memory] dialog box to close it.

TIP

Multiple images displayed can be shredded simultaneously.

1. Display the [Experiments in Memory] dialog box while showing multiple images in the [Display] panel.
2. Pressing down the Shift key, select multiple image files to be shredded.
3. Click the <Shredder> button.


<Shredder> button



4. Click the <Done> button in the [Experiments in Memory] dialog box to close.

2-3-4 Saving Comment Together with Image



<Experiment List> button

1. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.



Fig. 2-36 [Experiments in Memory] Dialog Box

2. In the [Experiments in Memory] dialog box, select the file name of the image to be saved with comment and click the <Comments> button. The [Image Comments] dialog box appears as shown below.

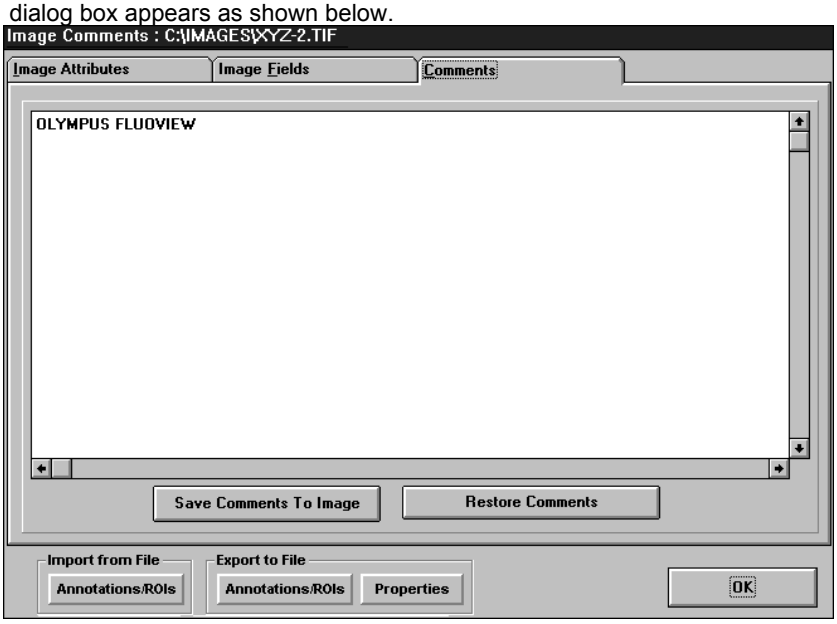


Fig. 2-37 [Image Comments] Dialog Box

3. Display the [Comments] panel at the front.

4. Enter comment from the keyboard.

NOTE **During modification of previously entered comment, if it is required to restore the originally entered comment, click the <Restore Comments> button. However, once the <Save Comments To Image> button is pressed, the original comment cannot be restored.**

5. Click the <Save Comments To Image> button.

NOTE **At the moment the <Save Comments To Image> button is clicked, the comment in the [Comments] panel is saved simply, but it is not yet saved in the image file. To save the comment in the image file, use the <Save> button in the [File I/O] panel.**

6. Click the <OK> button.
7. Click the <Done> button in the [Experiments in Memory] dialog box to close it.
8. Display the [File I/O] panel at the front.
9. Click the <Experiment> button in the [Save] group box.

TIP See section 2-3-1, “Saving Images” for details.

One Point!

The [Image Comments] dialog box can also be displayed by a mouse operation.

1. Display the image to be saved at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [Experiment Properties] from the menu.



2-3-5 Checking the Image Information/Acquisition Parameters



<Experiment List> button

1. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.



Fig. 2-38 [Experiments in Memory] Dialog Box

2. In the [Experiments in Memory] dialog box, select the file name of the image to check the image information and acquisition parameters and click the <Comments> button.
3. Display the [Image Attributes] panel at the front. The panel as shown below appears, where the information on the selected image is shown.

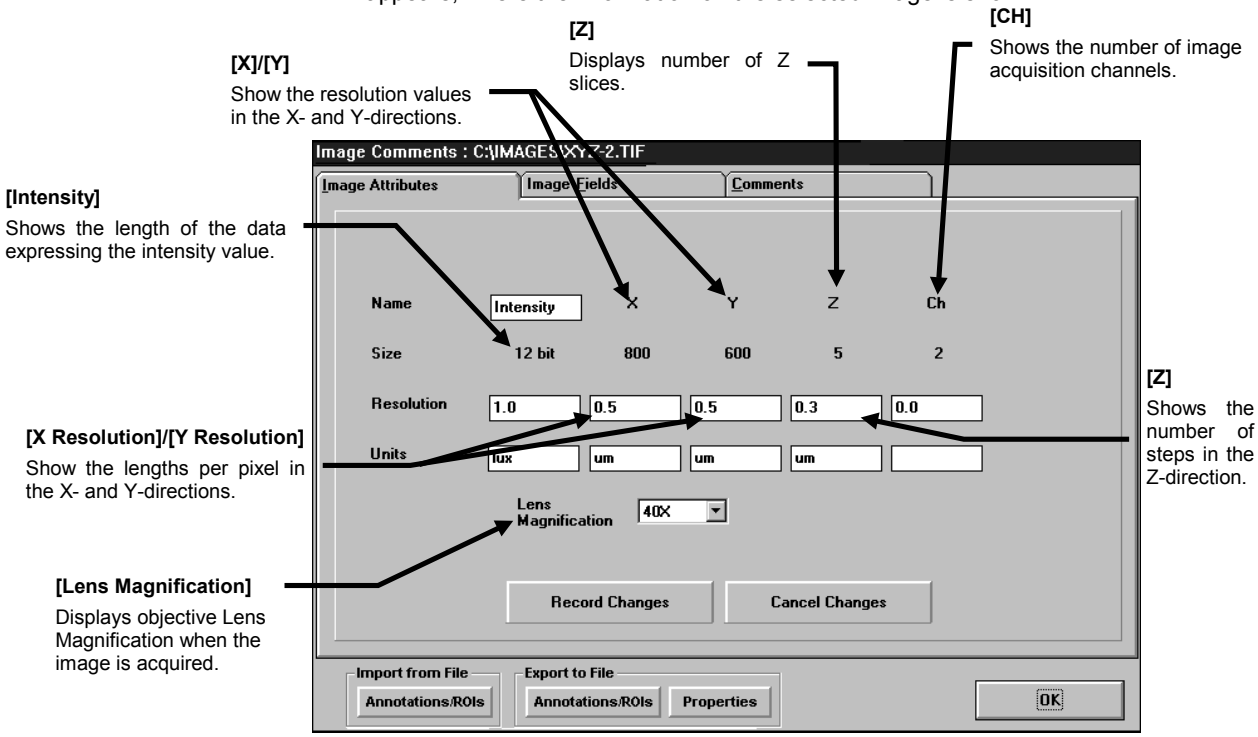


Fig. 2-39 [Image Attributes] Panel

TIP

The [X Size], [Y Size] and [Z Size] text boxes can also be used to change the lengths per image pixel or the number of steps in the Z-direction. These values are used in the scale display and many other analysis operations. When opening and analyzing an image file creased with another application on FLUOVIEW, enter the lengths per image pixel and the number of steps in the Z-direction if these values are known, then click the <Set Attributes> button.

NOTE

During modification of the lengths per image pixel, the number of steps in the Z-direction or the objective setting, if it is required to restore the previous setting, click the <Restore Attributes> button. However, once the <Set Attributes> button is pressed, the original setting cannot be restored.

NOTE

The objective setting can be changed even after having acquired the image. If the objective is not set with the [Acquire] panel before image acquisition, the analysis and measurement results of the image may be erroneous. In such a case, set the objective again.

NOTE

When the objective setting is changed, the lengths per image pixel will be re-calculated automatically and changed.

4. Display the [Image Fields] panel at the front. The panel as shown below appears, where the information on the selected image is shown.

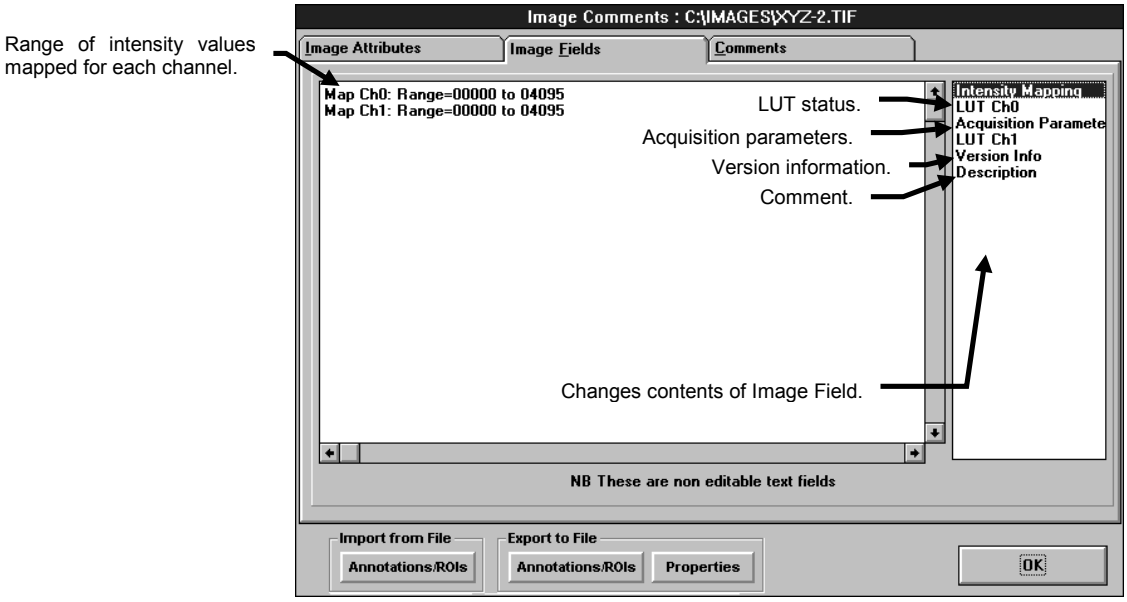
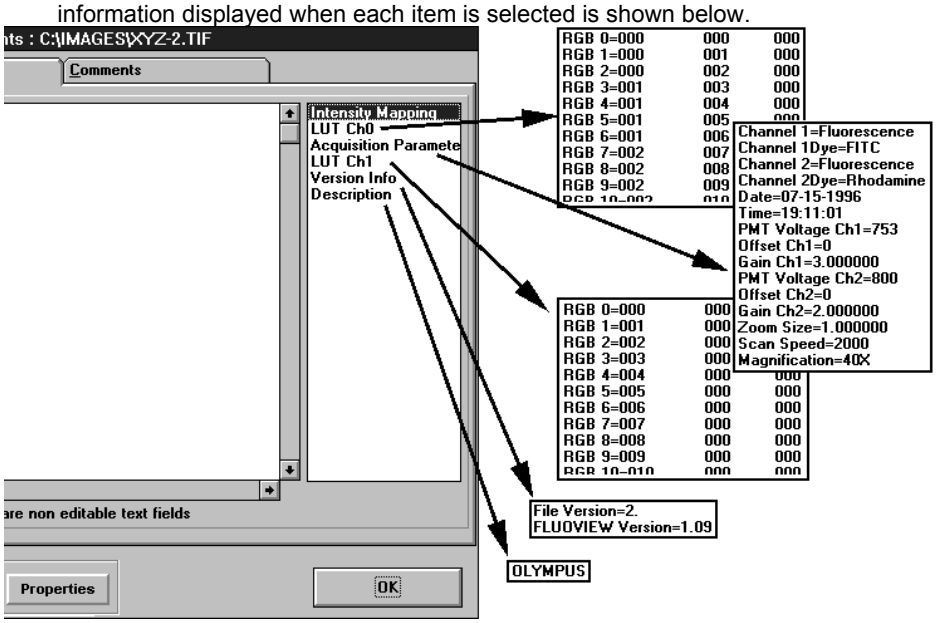


Fig. 2-40 [Image Fields] Panel

5. Select the information items to be checked from the list box on the right. The kind of information displayed when each item is selected is shown below.

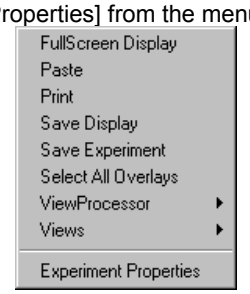


6. After checking the information, click the <OK> button.
7. Click the <Done> button in the [Experiments in Memory] dialog box to close it.

One Point!

The [Image Comments] dialog box can also be displayed by a mouse operation.

1. Display the image to be saved at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [Experiment Properties] from the menu.



2-3-6 Saving the Image Information/Observation Condition

The image information and observation condition can be saved as ASCII text file in a disk.

1. Display the [Display] panel of the image to be saved at the front position.

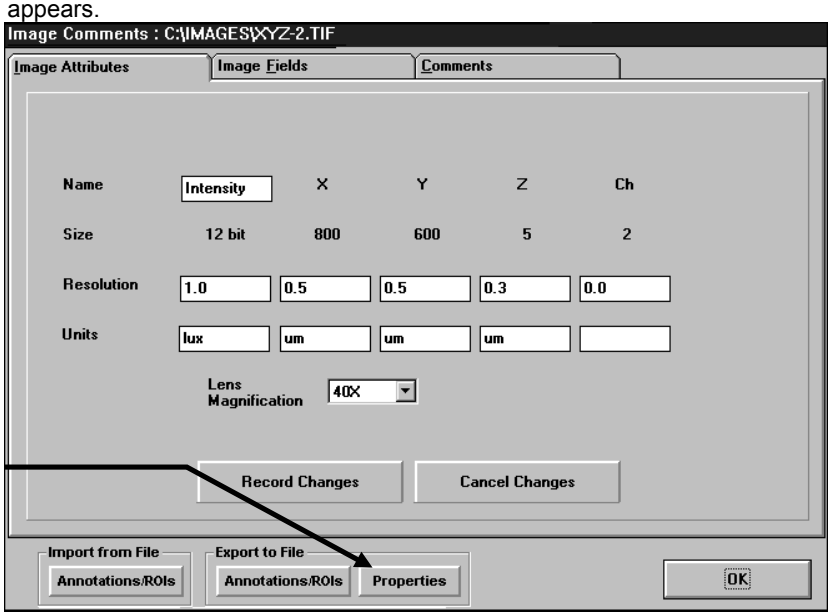

<Experiment List> button

2. Select the <Experiment List> button in the tool bar displayed at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box as shown below appears.



Fig. 2-41 [Experiments in Memory] dialog box

3. In the list in the [Experiments in Memory] dialog box, select the file name including the image whose image information and observation condition are to be saved. And click the <Comments> button. The [Image Comments] dialog box as shown below appears.



<Properties> button in the [Export to File] group box
Saves the image information and observation condition.

Fig. 2-42 [Image Comments] dialog box

4. Select the <Properties> button in the [Export to File] group box. The [Save to txt] dialog box as shown below appears.

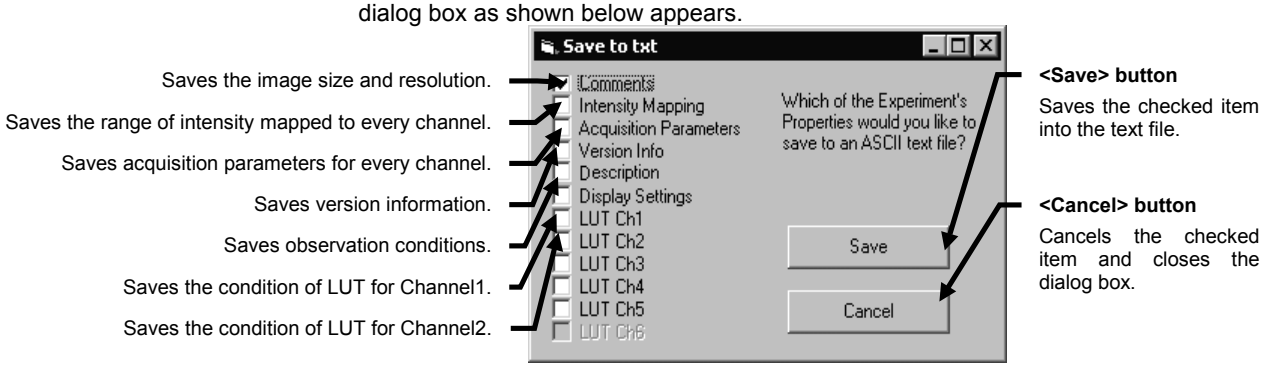


Fig. 2-43 [Save to txt] dialog box

TIP The [LUT Ch1] to [LUT Ch6] check boxes are displayed according to the channel used for image acquisition.

5. Check the check box of the item to be saved.

6. Select the <Save> button. The [Save As ASCII Text] dialog box as shown below appears.

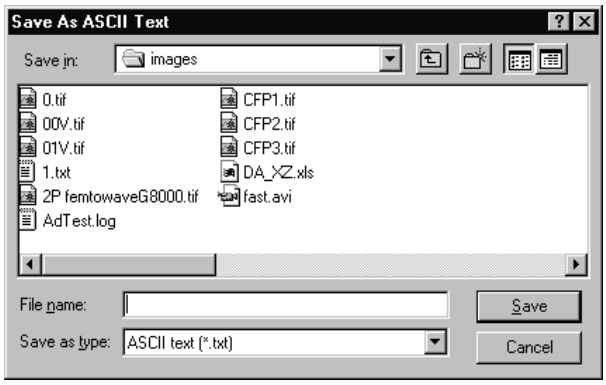


Fig. 2-44 [Save As ASCII Text] dialog box

7. In order to change the save destination drive or directory, use the [Save in:] drop-down list.

8. Confirm that “ASCII text (*.txt)” is selected in the [Save as type:] drop-down list.

9. Enter a file name into the [File Name:] text box.

10. Click the <Save> button.

NOTE **When the text file having the same name as the entered name is already exists, the dialog box appears to ask you to overwrite the existing file or not. When overwriting is not required, click the <NO> button and enter another name to save the file.**

TIP When the image to be saved was acquired through multiple channels, the observation conditions for all channels are saved.

2-3-7 Saving/Reading the Region File

The information on the shape, position, and color of certain region can be saved in a file and they can also be read.

2-3-7-1 Saving the Region File

1. Display the [Display] panel of the image including the region to be saved at the front position.


<Experiment List> button

2. Select the <Experiment List> button in the tool bar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box as shown below appears.



Fig. 2-45 [Experiments in Memory] dialog box

3. Select the image file name including the region to be saved in the [Experiments in Memory] dialog box and click the <Comments> button.

   The [Image Comments] dialog box as shown below appears.

<Annotations/ROIs> button in the [Export to File] group box
Saves the region into a file.

<Annotations/ROIs> button in the [Import from File] group box
Opens the file saving the region.

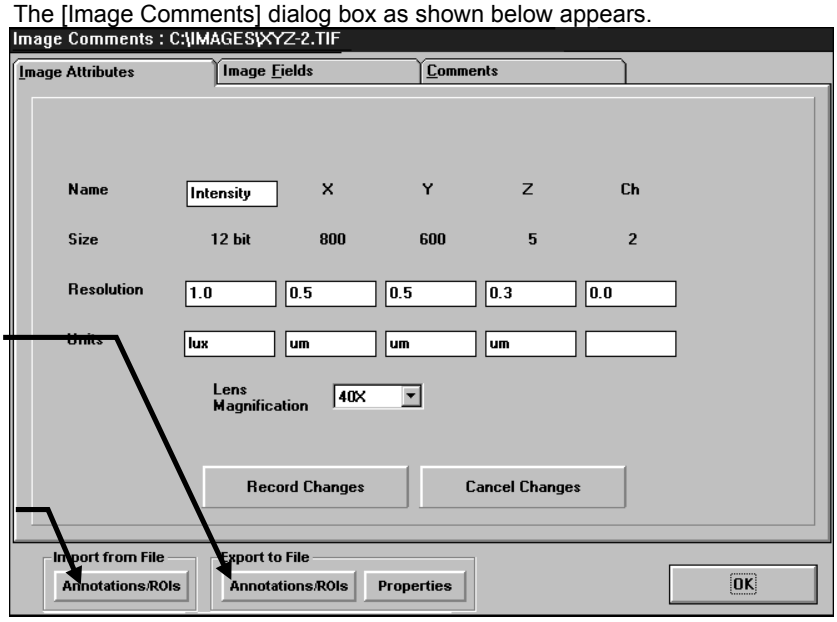


Fig. 2-46 [Image Comments] dialog box

4. Select the <Annotations/ROIs> button in the [Export to File] group box.

   The [Save Experiment As] dialog box as shown below appears.

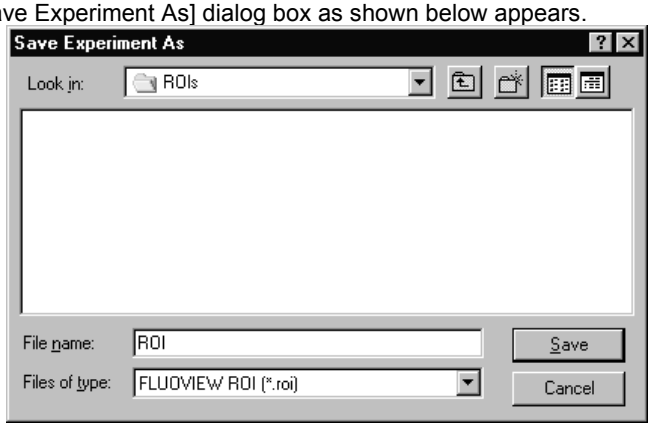


Fig. 2-47 [Save Experiment As] dialog box

5. Select the save destination drive or folder in the [Look in] drop-down list.

6. Select “FLUOVIEW ROI (*.roi)” in the [Files of type] drop-down list.

7. Enter the file name into the [File name] text box and click the <Save> button.

NOTE **When the text file having the same name as the entered name is already exists, the dialog box appears to ask you to overwrite the existing file or not. When overwriting is not required, click the <NO> button and enter another name to save the file.**

2-3-7-2 Reading the Region File

1. Display the [Display] panel of the image including the region to be read at the front position.


<Experiment List> button

2. Select the <Experiment List> button in the tool bar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box as shown below appears.



Fig. 2-48 [Experiments in Memory] dialog box

3. In the list in the [Experiments in Memory] dialog box, select the image file name including the region to be read and click the <Comments> button.
The [Image Comments] dialog box as shown below appears.

<Annotations/ROIs> button in the [Export to File] group box
Saves the region into a file.

<Annotations/ROIs> button in the [Import from File] group box
Reads the file saving the region.

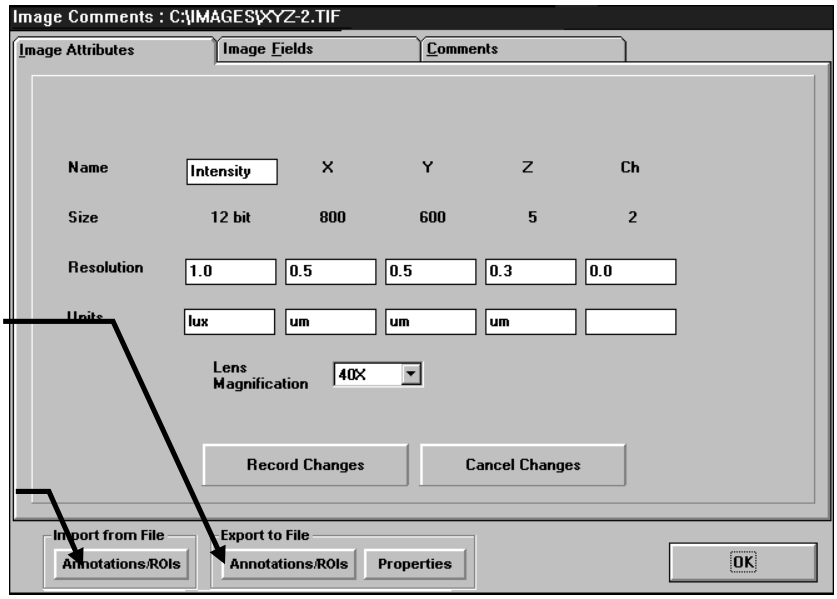


Fig. 2-49 [Image Comments] dialog box

4. Select the <Annotations/ROIs> button in the [Import from File] group box.
   The [Save Experiment As] dialog box as shown below appears.

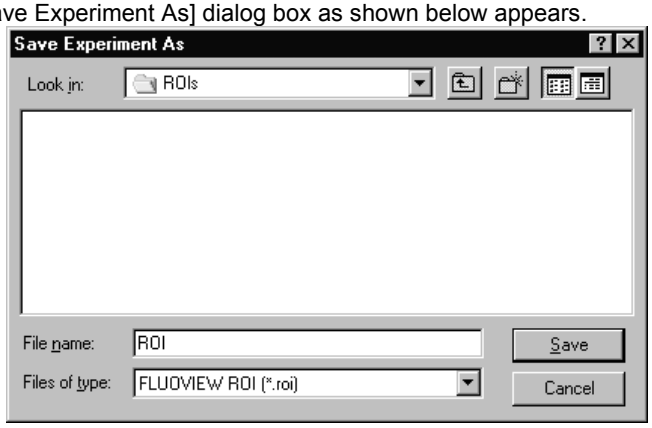


Fig. 2-50 [Save Experiment As] dialog box

5. Select the drive or folder where the file to be read is saved in the [Look in] drop-down list.

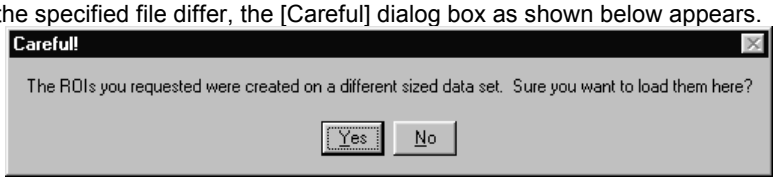
6. In the list below the [Look in] drop-down list, double-click the folder or sub-folder where the data to be read is saved to open.

7. Select “FLUOVIEW ROI (*.roi)” in the [Files of type] drop-down list.

8. Select the file to be read and click the <Open> button.

TIP

When the image size of the file including the region to be read and that of the specified file differ, the [Careful] dialog box as shown below appears.



Clicking the <OK> button reads the file in different image size, whereas clicking the <No> button cancels reading the region file.

When the region file is read in different image size, the upper left of the image is assumed as reference.

2-4 Changing the Image Display Method

The method of displaying an image acquired by observation or opened from a file can be changed as described below.

2-4-1 Displaying an Image in Simulated Colors


<LUT> button

1. Display the [Display] panel of the image to be colored at the front.
2. Click the <LUT> button in the toolbar at the bottom left of the screen. The [Color Tool] dialog box appears.

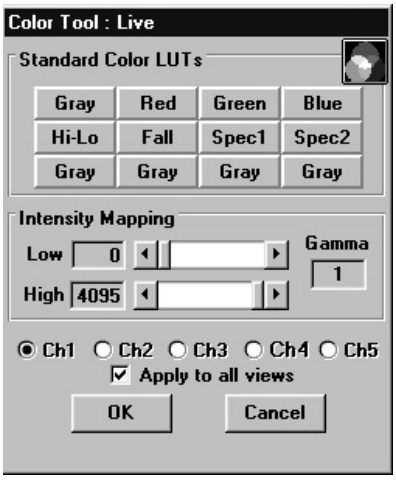


Fig. 2-51 [Color Tool] Dialog Box

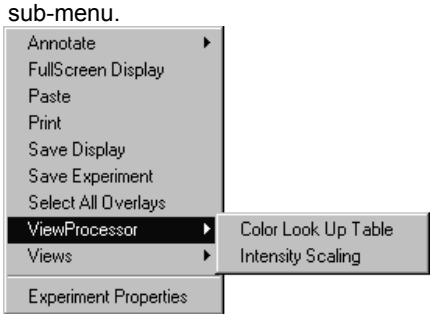
3. When the image was acquired from more than one channel, select the channels to be colored using the [Ch1], [Ch2], [Ch3], [Ch4] and/or [Ch5] option buttons. (The [Ch1], [Ch2] and [Ch3] option buttons are displayed only when an image acquired from more than one channel mode is displayed (selected).
4. From the [Standard Color LUTs] group box, select the desired color button. The selected LUT will be applied immediately to the image in the [Display] panel.
5. Click the <OK> button.

TIP The [Apply to all view] check box can be selected while the [Display] panel showing multiple images created in the [Title] panel is displayed.
When this check box is checked, all changes are applied to all of the images shown in the [Display] panel.

One Point!

The [Color Tool] dialog box can also be displayed by a mouse operation.

1. Display the image to be colored at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [View Processor] from the menu, then select [Intensity Scaling] from the displayed sub-menu.



2-4-2 Editing the LUT (Look Up Table)

An original LUT can be created by editing an existing LUT.

2-4-2-1 LUT Graph Editing According to Colors


<LUT> button


<Graph display> button

1. Display the [Display] panel of the image that you want to edit the LUT.
2. Click the <LUT> button in the toolbar at the bottom of the screen. The [Color Tool] dialog box appears as shown in Fig. 2-51.
3. Click the <Graph display> button on the top right of the [Color Tool] dialog box. The dialog box shows the LUT intensity graph.
4. Select the LUT color to be edited by checking the check boxes below the graph in the [Color LUT Tool] group box.
5. Set the range of intensity graph application using the [Low] and [High] scales in the [Intensity Mapping] group box.

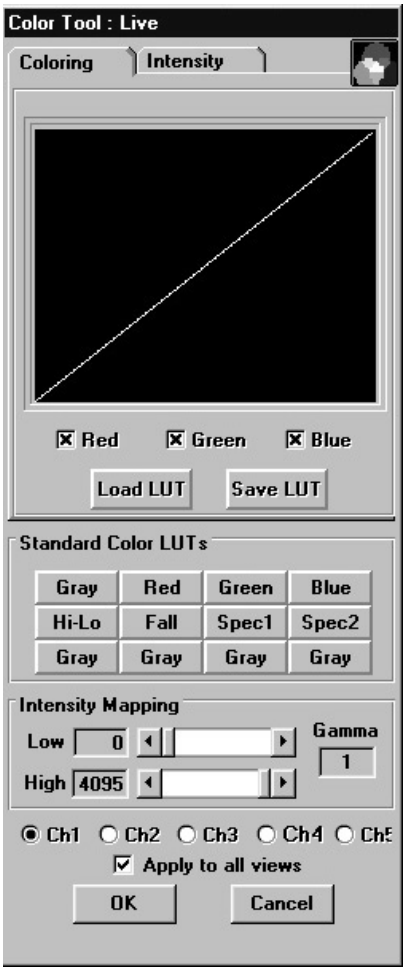


Fig. 2-52 LUT Intensity Graph Display

TIP

Double-clicked the [Low] scale, [High] scale and [Gamma] text box, they are reset to the default values (“0” with the [Low] scale, “4095” or “255” with the [High] scale and “1.0” with the [Gamma] text box).

6. The inclination of the graph in the [Color LUT Tool] group box can be changed by dragging an end of the graph line. The selected LUT will immediately be applied to the image in the [Display] panel.
7. If it is required to save the edited LUT in a file, click the <Save LUT> button in the [Color LUT Tool] group box.

TIP

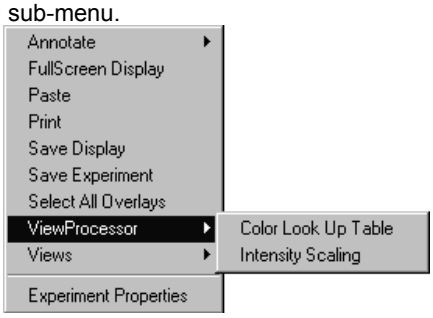
To load a previously saved LUT, click <Load LUT>.

8. Click the <Graph display> button at the top right of the [Color Tool] dialog box again.
9. Finally, click the <OK> button to exit from the LUT editing.

One Point!

The [Color Tool] dialog box can also be displayed by a mouse operation.

1. Display the image to be colored at the front of the [Display] panel, and right-click a point in the image.
2. A pop-up menu as shown below is displayed.
3. Select [View Processor] from the menu, then select [Intensity Scaling] from the displayed sub-menu.



2-4-2-2 LUT Graph Editing by Gamma Correction

The intensity data of an image can be reallocated to make it easier to view.


<LUT> button


<Graph display> button

1. Display the [Display] panel of the image to be subjected to LUT change.
2. Click the <LUT> button in the toolbar at the bottom left of the screen. The [Color Tool] dialog box appears as shown in Fig. 2-51.
3. Click the <Graph display> button on the top right of the [Color Tool] dialog box. The intensity graph of the LUT appears in the [Intensity] dialog box.
4. Set the range of intensity graph application with the [Low] and [High] scales in the [Intensity Mapping] group box.

TIP

Dragging one end of the graph makes it possible to change the inclination. The set intensity graph is immediately reflected in the image in the [Display] panel.

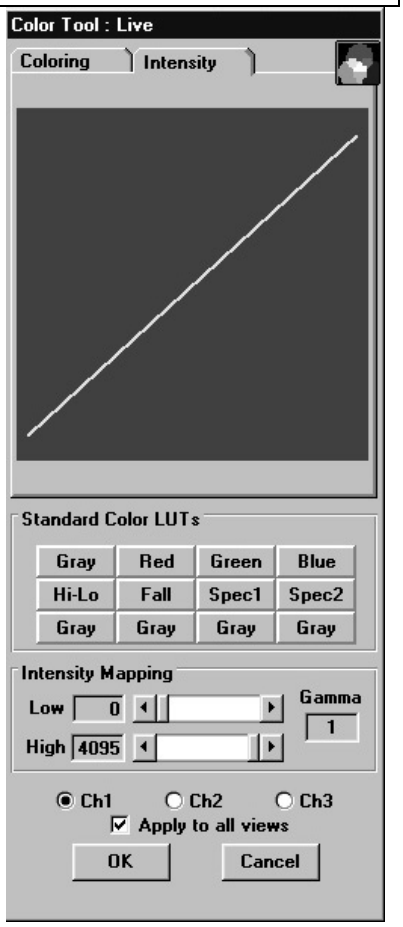


Fig 2-53 LUT Intensity Graph Display

TIP

Double-clicked the [Low] scale, [High] scale and [Gamma] text box, they are reset to the default values (“0” with the [Low] scale, “4095” or “255” with the [High] scale and “1.0” with the [Gamma] text box).

5. The gamma value can be changed by dragging on the graph. The set intensity graph is immediately reflected in the image in the [Display] panel.

TIP

The gamma value can also be changed by entering a value in the [Gamma] text box in the [Intensity Mapping] group box.

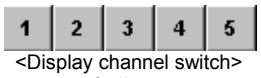
6. If it is required to save the LUT in a file, click the <Save LUT> button in the [Color LUT Tool] group box.

TIP To load a previously saved LUT, click the <Load LUT> button.

2-4-3 Switching the Displayed Channels (Ch1 – Ch5)

The buttons on the bottom left of the screen can be used to select where the image of a single channel or images of multiple channels are to be displayed. For the simultaneous display of multi-channel images, see section 2-4-4, “Displaying Images of Multiple Channels Simultaneously”.

1. Display the [Display] panel of for the image obtained from multiple channels at the front.
2. Click the image to display the <Display channel switch> buttons on the bottom left of the image.
3. Select the channels which should be displayed by pushing <Display channel switch> buttons. The color of a selected channel's button becomes darker.
4. Press the previously pressed <Display channel switch> buttons to let them disappear.



<Display channel switch> buttons



<Channel 1> button



<Channel 2> button



<Channel 3> button



<Channel 4> button



<Channel 5> button

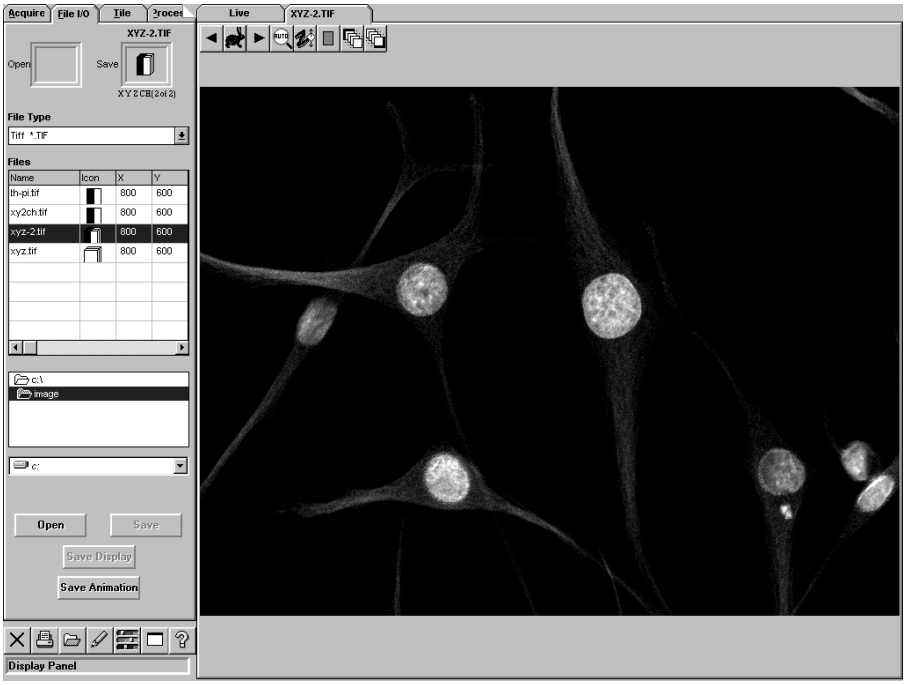


Fig. 2-54 Panel Displaying the Channel 2 Image

2-4-4 Displaying Images of Multiple Channels Simultaneously (Side By Side Views, Over And Under Views, Single View)

Images from multiple channels can be displayed either by merging them or placing them side by side. It is also possible to display the image of only one of these channels.

Use the buttons displayed at the top of the [Display] panel and those on the bottom right which are displayed when the corresponding image is clicked. For the display of the image of only one channel, see section 2-4-3, "Switching the Displayed Channels".

2-4-4-1 Displaying Images Separately Per Channel (Side By Side Views, Over And Under Views)

1. Display the [Display] panel of one of the images to be displayed side by side at the front.



<Display switching> button

2. Click the <Display switching> button at the top of the [Display] panel. The list of buttons as shown below appears.





<Side by side views> buttons



<Over and Under views> buttons

3. From the displayed list of buttons, click one of the <Side by side Views> button or <Over and under views> button. The icon shown in the <Display switching> button will change to the icons of the <Side by side views> button or <Over and under views> button.

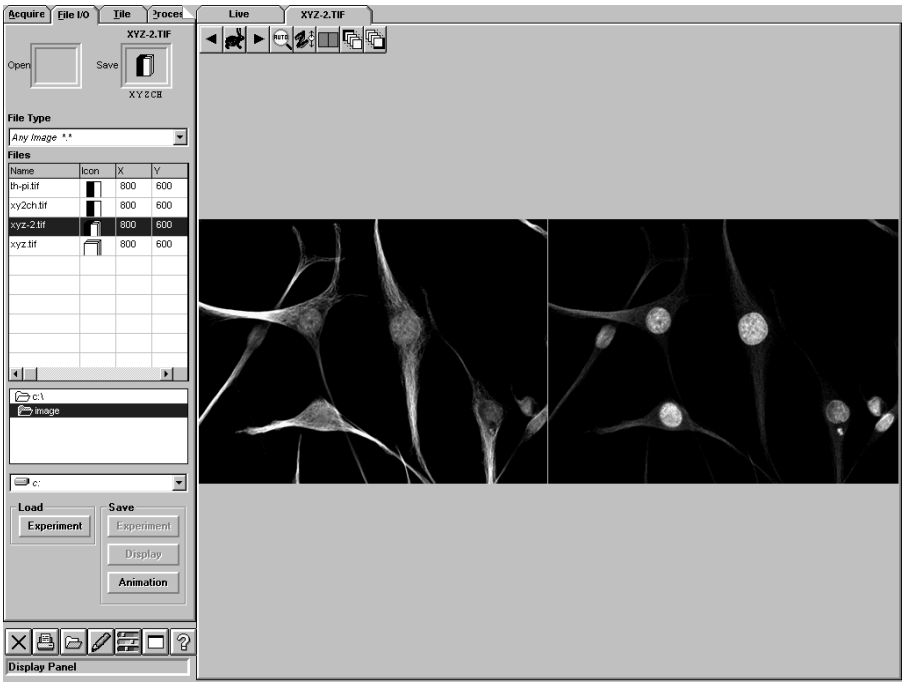


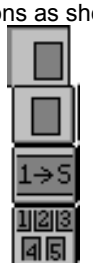
Fig. 2-55 Panel Displaying Images of Two Channels Side By Side

2-4-4-2 Displaying Merged Image of Multiple Channels (Single View)



<Display switching> button

1. Display the [Display] panel of the merged image of multiple channels at the front.
2. Click the <Display switching> button at the top of the [Display] panel. The list of buttons as shown below appears.





<Single View>button

3. From the displayed list of buttons, press the <Single View> button. The [Display] panel will show the images of multiple channels side by side. At the same time, the icon shown in the <Display switching> button will change to the icon of the <Single View> button.
4. Click the image to display the <Display channel switch> buttons at the bottom left of the image.



<Display channel switch> buttons



<Channel 1> button



<Channel 2> button



<Channel 3> button



<Channel 4> button



<Channel 5> button

5. Select the channels which should be displayed by pushing <Display channel switch> buttons. The color of a selected channel's button becomes darker.

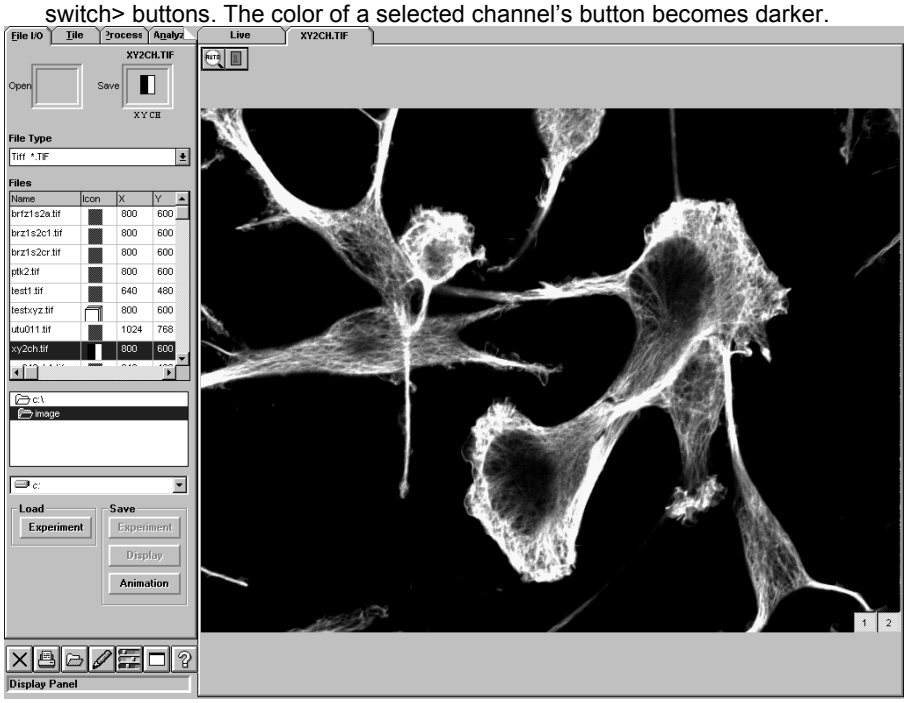


Fig. 2-56 Panel Displayed Merged Image of Two Channels

2-4-5 Changing the Number of Divided Images

The number of images viewed simultaneously can be changed.

TIP

Increased image is only to be displayed. The image increased in Add View is not subjected to these operations described below.

2-4-5-1 Increasing the Number of Divided Images

1. Display the [Display] panel of the image to be changed at the front.

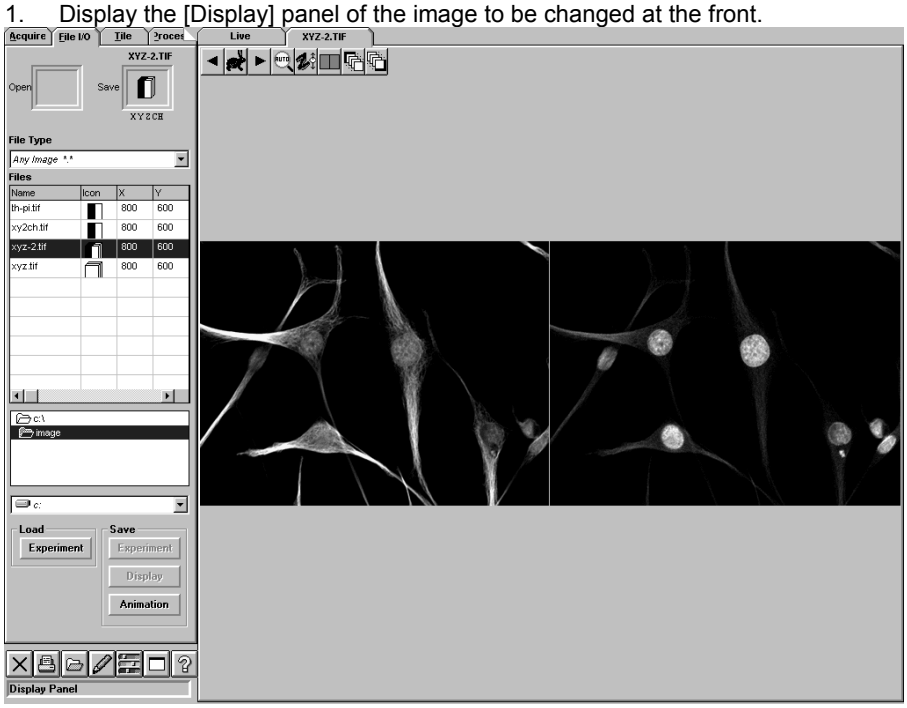


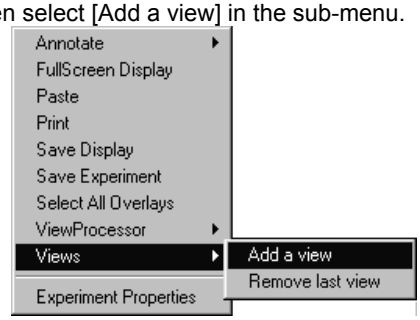
Fig. 2-57 [Display] panel

2. Right-click the image.

   A pop-up menu as shown below appears.



3. Select [Views], then select [Add a view] in the sub-menu.



4. A view is added to the rightmost position on the [Display] panel.

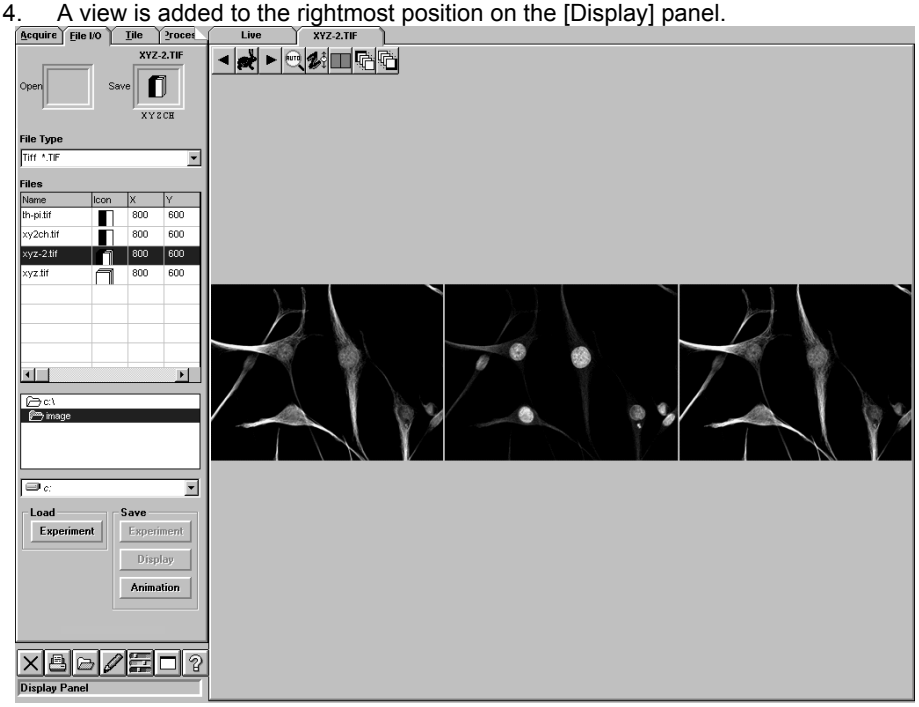


Fig. 2-58 [Display] Panel After View Addition

TIP Up to 6 views can be displayed at once.

2-4-5-2 Decreasing the Number of Divided Images

1. Display the [Display] panel of the image to be changed at the front.

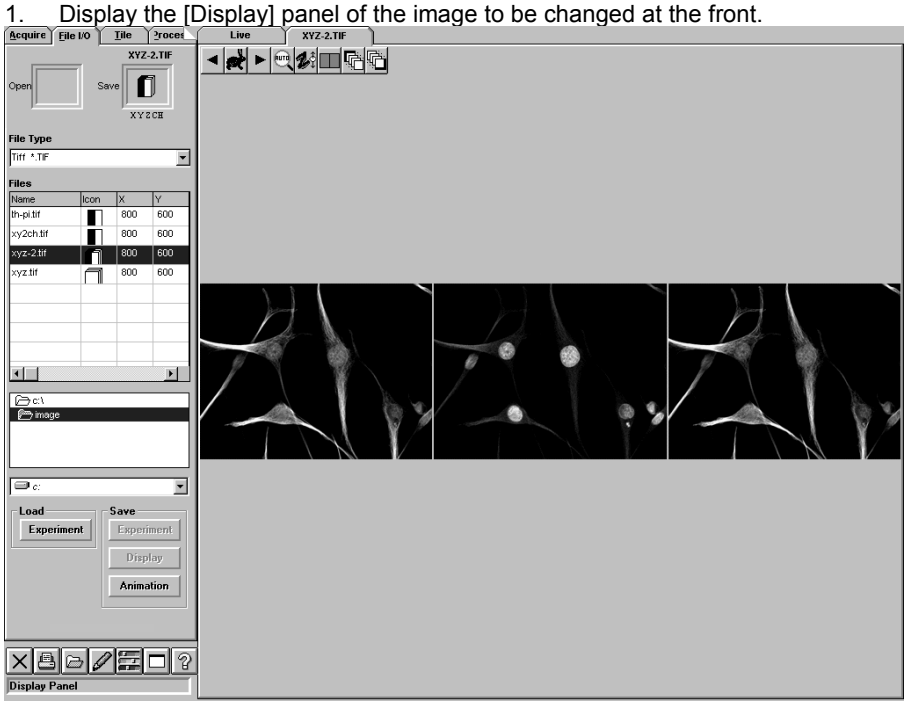


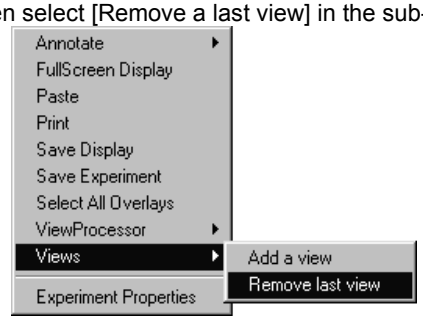
Fig. 2-59 [Display] panel

2. Right-click the image.

   A pop-up menu as shown below appears.



3. Select [Views], then select [Remove a last view] in the sub-menu.



4. A view is removed from the [Display] panel.

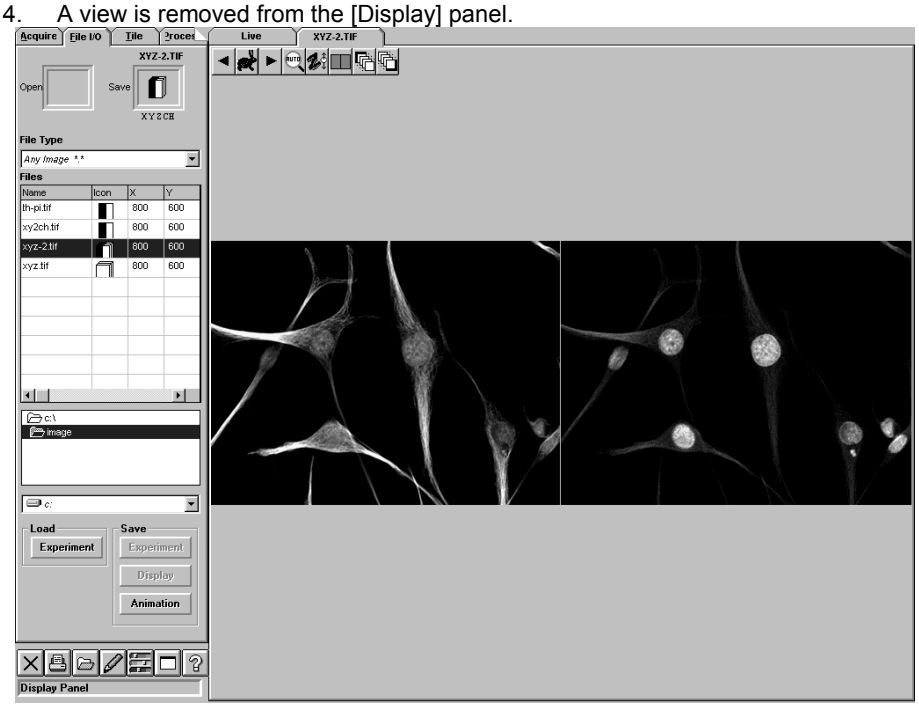
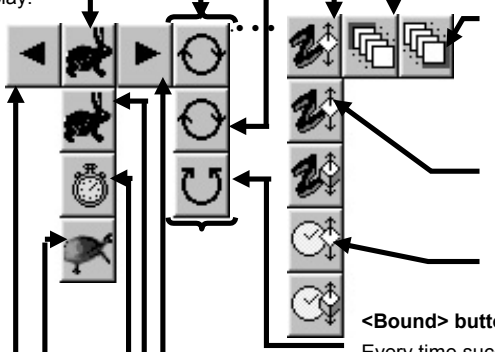


Fig 2-60 [Display] panel after View Removal

2-4-6 Switching the Display Method of Multiple Images

With images composed of multiple slices, such as time-lapse images or images acquired by changing the multiple sections, the image to be displayed at the front position can be switched or the images can be displayed successively.

1. Display the [Display] panel of the multiple images.
   The buttons as shown below are displayed at the top of the [Display] panel.
2. To switch the image to the image of another multiple sections, click the <Z/T series switch> button then, from the displayed list of buttons, click the <XYZ series> button.
   To switch the image to the image of another moment in the elapsed time, click the <Z/T series switch> button then, from the displayed list of buttons, click the <XYT series> button. (The icon in the <Z/T series switch> button will change to the icon of the selected button.)
3. Display the image to be displayed at the front by using the <Display> buttons at the top of the [Display] panel.



<Loop> button
Successive display or frame-by-frame display is repeated in the same direction.

<Loop/Bound switch>button

<Display speed switch>button
Set the speed of successive display.

<Z/T series switch> button

<Set start position> button
When successive display or frame-by-frame display is required, set the image with which the display should start. (“0” is set when this button is not used.)

<Set end position> button
When successive display or frame-by-frame display is required, set the image with which the display should end. (Assuming that the number of images is n, “n-1” is set when this button is not used.)

<XYZ series> button
Displays one of multiple images by selecting it according to the multiple sections (Z).

<XYT series> button
Displays one of multiple images by selecting it according to the time (T).

<Bound> button
Every time successive display or frame-by-frame display in a direction ends at the start or end position, the display is restarted in the opposite direction.

<Display>button
Press to start display of n-slices of image from the start position to the end position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame

<Rabbit>button
Press for successive display of image slices without pause.

<Clock>button
Press for successive display of image slices at the time interval set in the [Time Series] sub-panel.

<Turtle>button
Press for successive display of image slices at an interval of 0.8 sec

<Display>button
Press to start display of n-slices of image from the end position to the start position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

TIP

Click and hold the <Display> button for successive display. To stop it, click the <Display> button again.

For frame-by-frame display, simply click the <Display> button.

2-4-7 Displaying Multiple Image Slices Together

With images composed of multiple slices, such as time-lapse images or images acquired by changing the multiple sections, the image slices can be displayed together for simultaneous viewing. However, note that the size per image reduces when the number of displayed image slices increases.

Use the [Tile] panel for displaying images together.

Display the [Tile] panel.

Icon of the displayed images (image slices shown together).

[Tiling] group box

Shows how the images are displayed in the [Display] panel.

[Columns] text box

Sets the number of columns, or the number of image slices displayed in a horizontal row.

[Experiment]

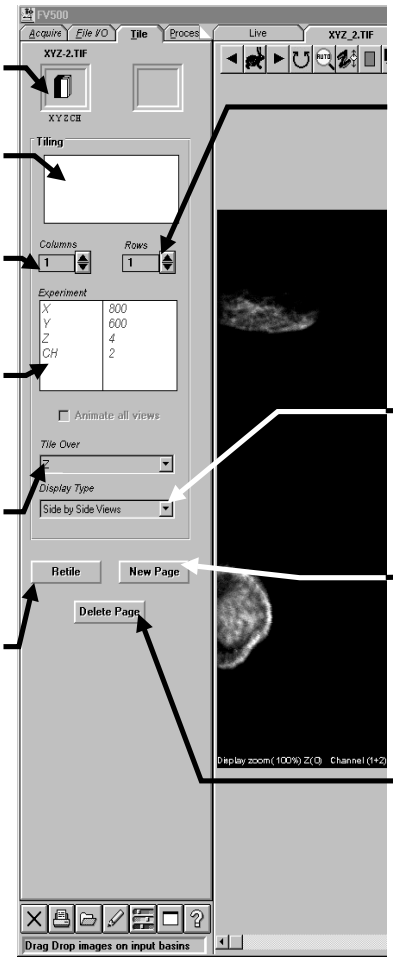
Shows the acquisition parameters used in acquisition of the displayed images.

[Tile Over] drop-down list

Select the acquisition parameter to be based on when arranging the images. [Self], [Z] or [T] can be selected.

<Retile> button

Displays the images by arranging them in the currently displayed [Display] panel.



[Rows] text box

Sets the number of rows, or the number of image slices displayed per vertical column.

[Display Type] drop-down list

Select the display method. [Single View], [Side by Side Views] or [Over and Under Views] can be selected.

[New Page] button

Displays images by arranging them in a new [Display] panel.

[Delete Page] button

Deletes the [Display] panel being displayed.

Fig. 2-61 [Tile] panel

2-4-7-1 Displaying Multiple Images Per Channel

1. Display the [Display] panel of one the images which are to be displayed together. The icon of the image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in image acquisition are displayed in the [Experiment] panel.
2. Set the number of images to be displayed together by using the <▲> and <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.
3. When there are multiple images to be displayed, select the following items in the [Tile Over] drop-down list.
   - Self: The same images as the image being displayed will be displayed.
   - Z: Images are displayed according to change in multiple sections.
   - T: Images are displayed according to change in time.
4. Select the display method from the [Display Type] drop-down list.
5. Click the <New Page> button. A new [Display] panel appears showing the images displayed per channel.



Use the <Retile> button when it is required to re-arrange the images in the currently displayed [Display] panel.

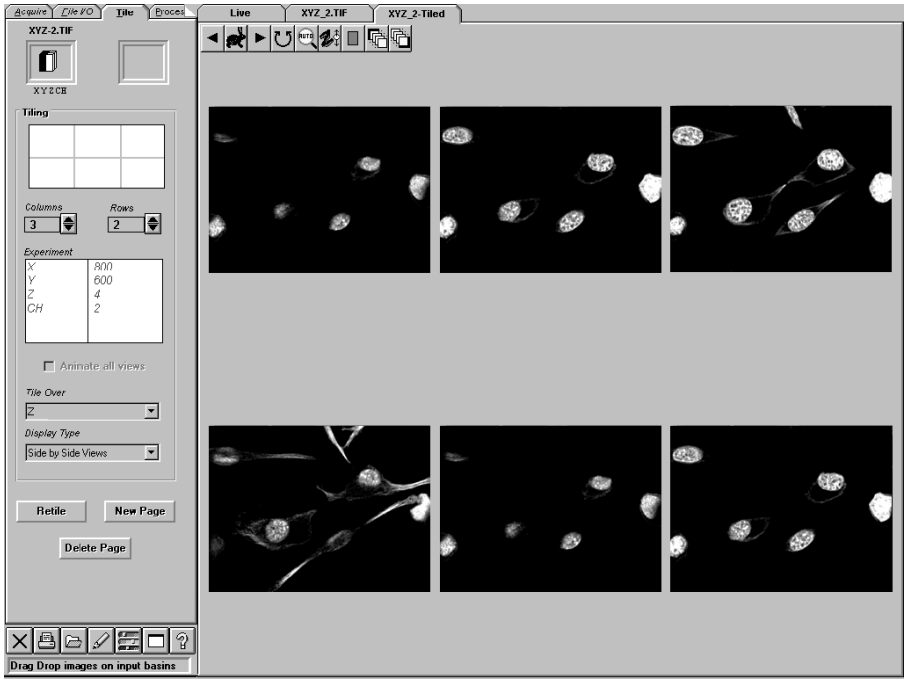


Fig. 2-62 Panel Displaying Images Per Channel

2-4-7-2 Displaying Images of Two Channels Together

Images acquired in a multi-channel mode can be displayed together for simultaneous view.

The operation method is identical to the method for displaying images per channel except for the following point. See section 2-4-7-1, “Displaying Multiple Images Per Channel”.

- With images acquired in a multi-channel mode, select the display method from the [Display Type] drop-down list.

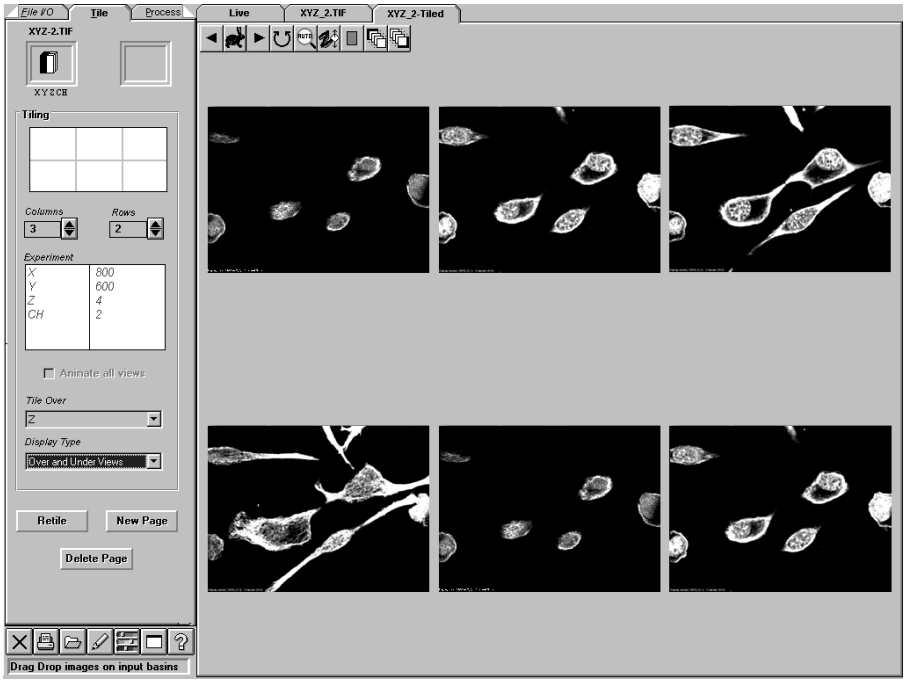


Fig. 2-63 Panel Displaying Images by Overlaying Different Channels

2-4-7-3 Displaying Time-Lapse Images

Multiple images acquired over time can be displayed side by side for simultaneous view.

1. Display the [Display] panel of one of the time-lapse images to be displayed together.
2. Set the number of images to be displayed together by using the <▲> and <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.
3. Select [T] from the [Tile Over] drop-down list.
4. When the time-lapse images were acquired in a multi-channel mode, select the display method from the [Display Type] drop-down list.
5. Click the <New Page> button. A new [Display] panel appears showing the images displayed per channel.

NOTE **Use the <Retile> button when it is required to re-arrange the images in the currently displayed [Display] panel.**

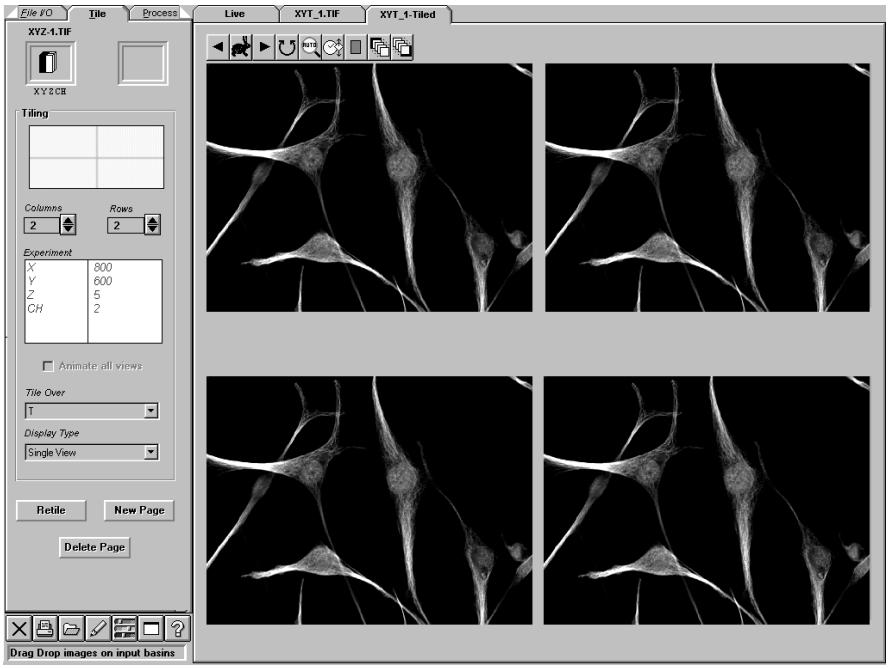


Fig. 2-64 Panel Showing Time-Lapse Images Together

2-4-7-4 Displaying Multiple Multiple sections Images

Images acquired from different multiple sections can be displayed together for simultaneous view.

The operation method is identical to the method for displaying time-lapse images except for the following point. See section 2-4-7-3, “Displaying Time-Lapse Images”.

- Select [Z] from the [Tile Over] drop-down list.

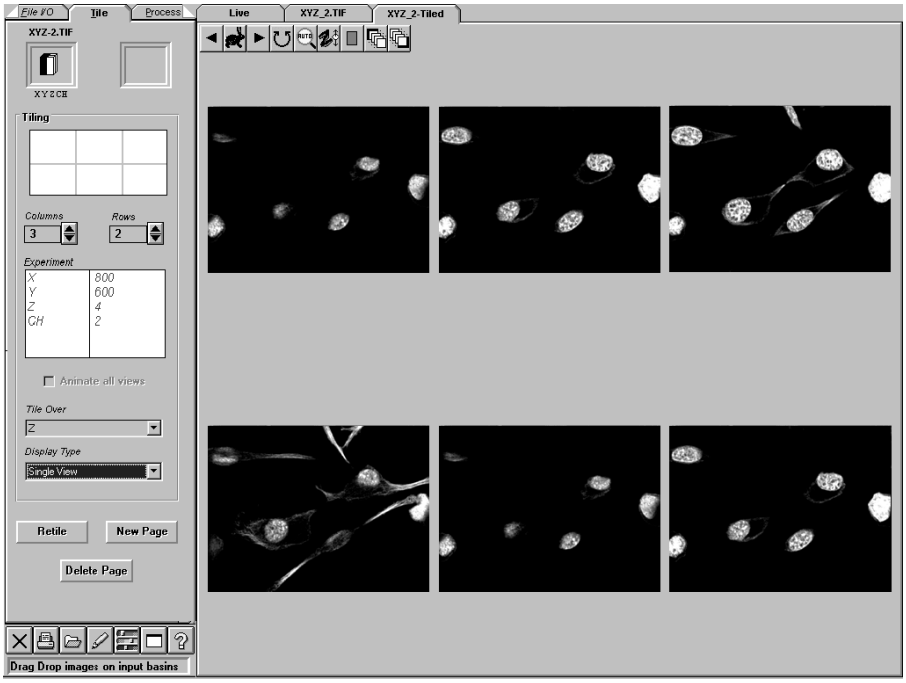


Fig. 2-65 Panel Displaying Different Multiple sections Images Together

2-4-7-5 Displaying Same Images in Different Display Methods

Images composed of multiple image slices can be displayed in more than one display methods together.

1. Display the [Display] panel of one of the images (image composed of multiple slices) to be displayed together.

   The icon of the image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in image acquisition are displayed in the [Experiment] panel.
2. Set the number of images to be displayed together by using the <▲> or <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.
3. Select [Z] or [T] from the [Tile Over] drop-down list.

4. Click the <New Page> button. A new [Display] panel appears showing the same images as the displayed image. The number of the displayed images is as set in step 2 above.

NOTE **Use the <Retile> button when it is required to re-arrange the images in the currently displayed [Display] panel.**

5. Click the top left image of the displayed images.
   Buttons appear above the clicked image.
6. Switch the image display using the displayed buttons.

TIP For details on the display switching method, see sections 2-4-3, “Switching the Displayed Channels”, 2-4-4, “Displaying Images of Multiple Channels Simultaneously” and 2-4-6, “Switching the Display Method of Multiple Images”.

7. Perform steps 5 and 6 above for each of the displayed images.

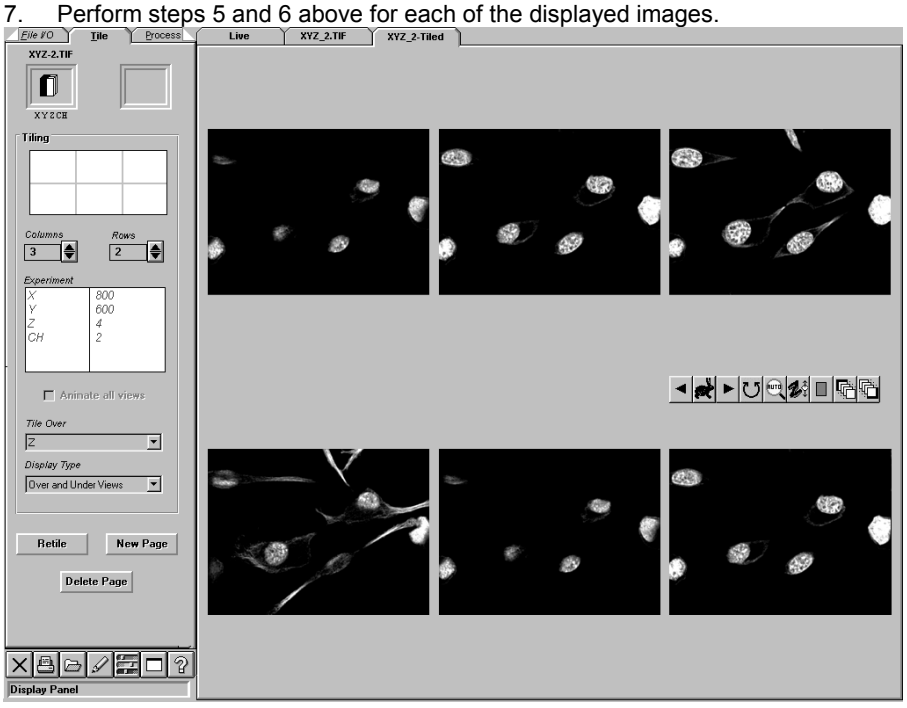


Fig. 2-66 Panel Showing 2-Channel Mode Images in Different Display Methods

Fig. 2-66 shows a panel where the display methods are varied as shown below.



2-4-7-6 Re-arranging Images Using the Same Display Method

All of the images displayed together in a [Display] panel can be rearranged simultaneously based on the same display method (channels, magnification, scroller position).

1. Click one of the images displayed together.
   Two sets of buttons appear above and below the clicked image.
2. Change the image display method using the displayed buttons.
3. Click the <Retile> or <New Page> button. All of the images in the panel are re-displayed using the same display method as that set in step 2 above.

2-4-7-7 Displaying Different Images Together

Two completely different images can be displayed together.
The two images to be displayed together should be acquired by observation or loaded by opening a file. If two images are not available, prepare them by image acquisition or file opening.

1. Display the [Display] panel of either image to be displayed with another image.
   The icon of the image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in image acquisition are displayed in the [Experiment] panel.



<Experiment List> button

2. Click the <Experiment List> button in the toolbar at the bottom of the [Tile] panel. The [Experiments in Memory] dialog box appears as shown below.

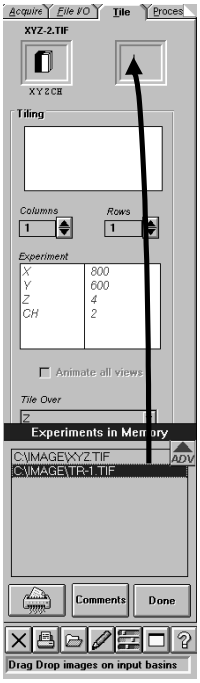




Fig. 2-67 [Experiments in Memory] Dialog Box

3. From the [Experiments in Memory] dialog box, select the file name of the second image to be displayed and drag it into the frame at the top right of the [Tile] panel. The icon of the second image is displayed in the frame at the top left of the [Tile] panel and the acquisition parameters used in the image acquisition are displayed in the [Experiment] panel.

TIP The mouse pointer turns into the image icon during dragging.

4. Click the <Done> button in the [Experiments in Memory] dialog box to close it.
5. Set the number of images to be displayed together by using the <▲> and <▼> buttons in the [Columns] and [Rows] text boxes. How the images will be arranged can be confirmed in the gray box at the upper part of the [Tiling] group box.
6. When there are multiple images to be displayed, select the following items in the [Tile Over] drop-down list.
   - Self: The same images as the image being displayed will be displayed.
   - Z: Images are displayed according to change in multiple sections.
   - T: Images are displayed according to change in time.

7. When displaying images acquired in a multi-channel mode, select the display method from the [Display Type] drop-down list.
8. Click the <New Page> button. A new [Display] panel appears showing the two images one above the other.

   The image of the file displayed in the frame at the top left of the [Tile] panel is displayed on the upper part of the [Display] panel, and that of the file displayed in the frame at the top right is displayed on the lower part.

NOTE **Use the <Retile> button when it is required to re-arrange the images in the currently displayed [Display] panel.**

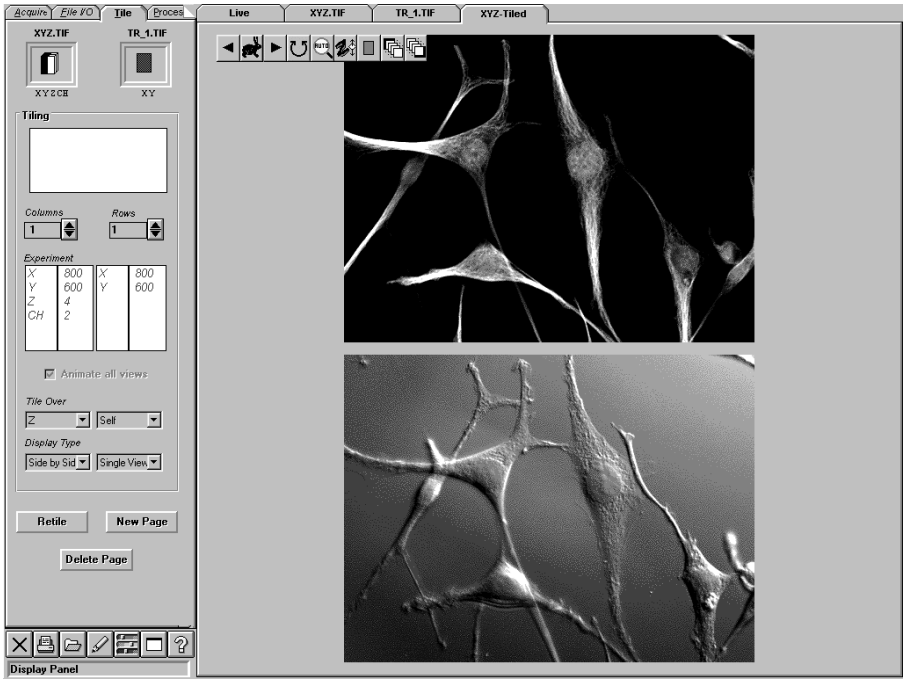


Fig. 2-68 Panel Displaying Two Different Images Together

2-4-8 Displaying an Image in Full Screen

Only the image itself can be displayed to fill the screen by erasing all other display components such as the toolbar, panel and status bar. This feature is useful for taking pictures using an analog printer for creation of a slide.


<Full Screen> button

1. Display the [Display] panel of the image to be displayed.
2. Click the <Full Screen> button in the toolbar at the bottom left of the screen.

TIP

When the mouse left button is clicked while the image is displayed full screen, the buttons which are usually displayed on the top of the [Display] panel (<Display> buttons, etc.) and those usually displayed on the bottom right (<Display channel switch> button) can be displayed in the image.

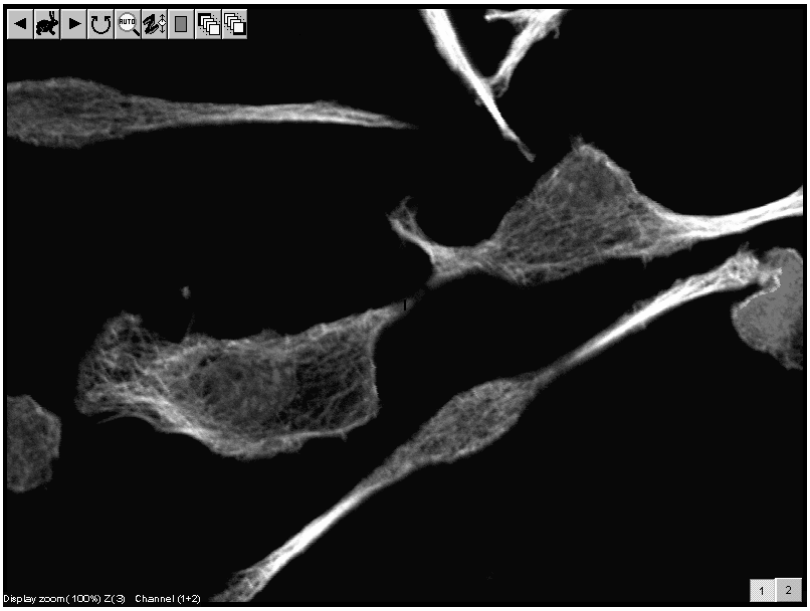


Fig. 2-69 Panel Showing the <Display channel switch>, <Display> and Other Buttons

3. Click the mouse right button on the image to cancel the full-screen display.

One Point!

The image can also be displayed full screen by the mouse operation on the image.

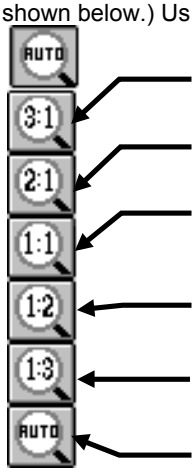
1. Display the [Display] panel of the image to be displayed full screen, and click the mouse right button on the image.
2. A pop-up menu as shown below appears.
3. Select [FullScreen Display] from the.



2-4-9 Magnifying/Reducing an Image

The image can be magnified or reduced using the buttons displayed at the top of the [Display] panel. Magnification or reduction up to 3:1 or 1:3 the original image is possible.

1. Display the [Display] panel of the image to be magnified or reduced.
2. The buttons as shown below are displayed on the top of the [Display] panel. (Usually, the <Auto> button is displayed, and clicking it displays the list of buttons shown below.) Us these buttons to magnify or reduce the image.



<3:1> button
Magnifies the original image by 3 times.

<2:1> button
Magnifies the original image by 2 times.

<1:1> button
Displays the image in the original size.

<1:2> button
Reduces the original image by 1/2.

<1:3> button
Reduces the original image by 1/3.

<AUTO> button
Magnifies or reduces the image according to the current [Display] panel size.

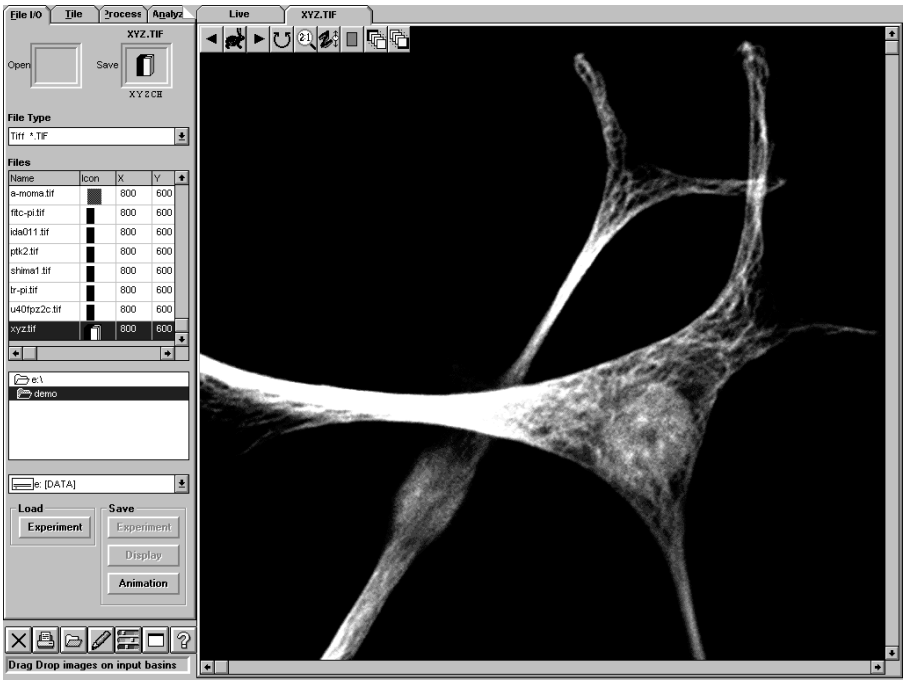


Fig. 2-70 Panel Showing a 2:1 Magnified Image

2-5 Image Processing

Images can be processed using the [Process] panel. Display the [Process] panel at the front.

2-5-1 Filtering

Use the [Filters] sub-panel in the [Process] panel to apply filtering to images.

1. Display the [Filters] sub-panel in the [Process] panel at the front.

Icon of the image being displayed (Image to be processed by filtering).

[Smoothing Filters] group box
Provides a command button for each noise filter.

[Sharpening Filters] group box
Provides a command button for each of the contour enhancement and image sharpening filters.

[DIC Image Filter] group box
Used for correction of DIC level irregularities.

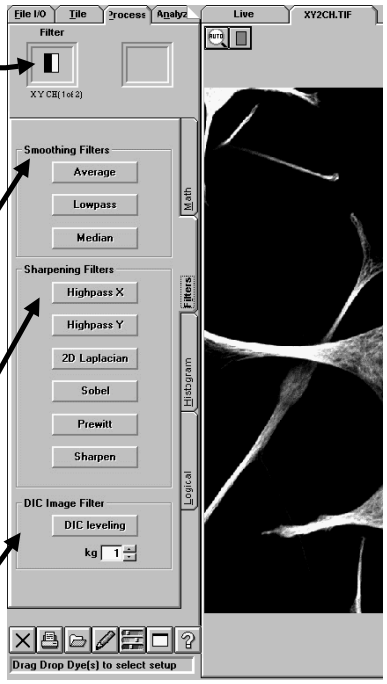


Fig. 2-71 [Filters] Sub-panel

2-5-1-1 Contour Enhancement

When an image is blurred by the boundaries between image grains becoming unclear, it can be sharpened by applying contour enhancement. Five types of filters are provided for use in the contour enhancement.

1 Laplacian filter

This filter enhances the contours of the image grains. If the image contains noise, the noise is also enhanced. By adding the original image to the image processed with Laplacian filtering, it is possible to obtain an image with stronger contour enhancement. The filter format is as shown below.

$$\begin{pmatrix} -1 & -1 & -1 \\ -1 & 8 & -1 \\ -1 & -1 & -1 \end{pmatrix}$$


<Set start position> button


<Set end position> button


<Display>button



<Display channel switch> buttons



<2D Laplacian> button

1. Display the [Display] panel of the image to be processed with Laplacian filtering.
2. When the image is composed of multiple image slices, the range of image slices to be filtered can be specified using the <Set start position> and <Set end position> buttons above the image. First display the image slice to start filtering using the <Display> button and click the <Set start position> button. Then, set the image slice to end filtering in the same way as above.
3. When the image to be filtered was acquired in the multi-channel mode, filtering is applied only to the channels being displayed.
   Example) When only the Ch1 image is displayed, filtering is applied to the Ch1 image only.

TIP For the switching of channels, see section 2-4-3, “Switching the Display Channels”.

4. Click the <2D Laplacian> button. A new [Display] panel having the page tab named [Filter] appears, showing the filtered image.

TIP During filtering, the status bar shows the progress of processing.

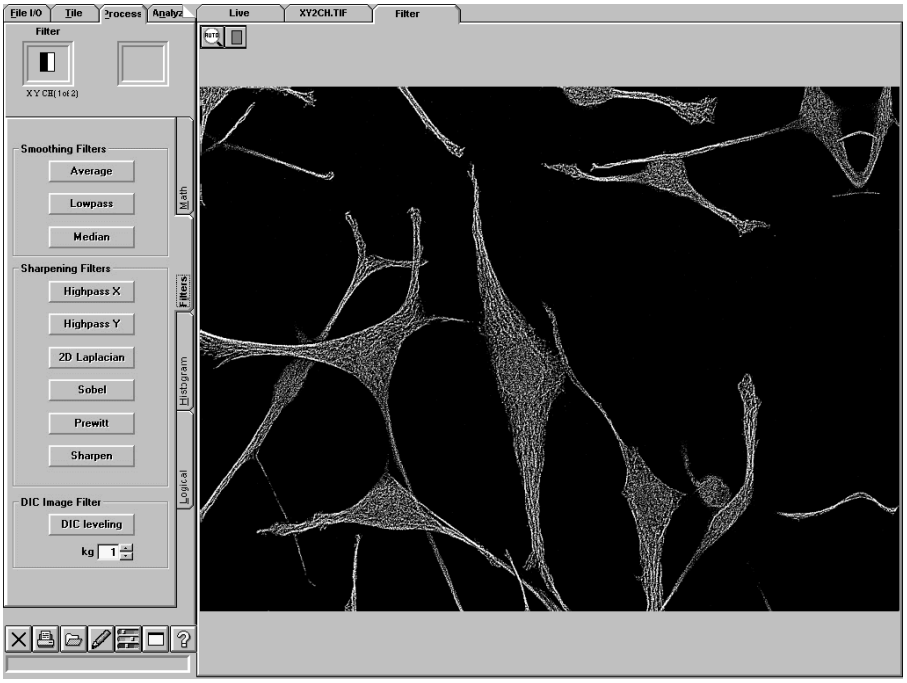


Fig. 2-72 Panel Displaying the Laplacian Filtered Image

2 Sobel filter

This filter enhances the contours of the image grains. If the image contains noise, the noise is also enhanced. It has two filter formats, X and Y, as shown below. The format providing the larger value after filtering is used.

$$\begin{pmatrix} -1 & -2 & -1 \\ 0 & 0 & 0 \\ 1 & 2 & 1 \end{pmatrix} \qquad \begin{pmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{pmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-5-1-1-1, “Laplacian filter”.



<Sobel> button

- Click the <Sobel> button.

3 High-pass X filter

The HIGH-PASS X filter passes the high-frequency structures in the X-direction of image. In this way, it can extract details by detecting positions with large variation. This processing is useful for making structures clear or extract the edges. The filter format is as shown below.

$$\begin{pmatrix} -1 & -1 & -1 \\ 0 & 0 & 0 \\ 1 & 1 & 1 \end{pmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-5-1-1-1, “Laplacian filter”.



<Highpass X> button

- Click the <Highpass X> button.

4 Highpass Y filter

The HIGH-PASS Y filter passes the high-frequency structures in the Y-direction of image. In this way, it can extract details by detecting positions with large variation. This processing is useful for making structures clear or extract the edges. The filter format is as shown below.

$$\begin{pmatrix} 1 & 0 & -1 \\ 1 & 0 & -1 \\ 1 & 0 & -1 \end{pmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-5-1-1-1, “Laplacian filter”.



<Highpass Y> button

- Click the <Highpass Y> button.

5 Prewitt filter

This filter enhances the contours of image grains in a similar way to the Sobel filter, but more strongly than it. It has two filter formats, X and Y, as shown below. The format providing the larger value after filtering is used.

X

$$\begin{pmatrix} -2 & -2 & -2 \\ 0 & 0 & 0 \\ 2 & 2 & 2 \end{pmatrix}$$

Y

$$\begin{pmatrix} -2 & 0 & 2 \\ -2 & 0 & 2 \\ -2 & 0 & 2 \end{pmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-5-1-1-1, “Laplacian filter”.



<Prewitt> button

- Click the <Prewitt> button.

2-5-1-2 Noise Reduction

When random noise interferes with an image, its irregularity increases and it become harder to see. Such noise can be reduced by means of filtering. Three kinds of filters are available for noise reduction.

1 Averaging filter

The averaging filter is used to eliminate details in image or reduce noise. However, as it makes everything in an image smooth, it also makes the edge sections dull, which sometimes result in the image resolution deterioration. The filter format is as shown below.

$$\begin{pmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{pmatrix} \text{ X } 1/9$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-5-1-1-1, “Laplacian filter”.



<Average> button

- Click the <Average> button.

2 Median filter

The Median filter reduces noise in image while leaving the edges intact. However, it may be ineffective in case noise is concentrated in some positions or the image is very noisy.

The operation method is identical to Laplacian filtering except for the following point. See section 2-5-1-1-1, “Laplacian filter”.



<Median> button

- Click the <Median> button.

3 Low-pass filter

The low-pass filter passes the low-frequency structures. In this way, it can eliminate small grains and provide smooth, noise-reduced image. filter format is as shown below.

$$\begin{bmatrix} 1 & 2 & 1 \\ 2 & 4 & 2 \\ 1 & 2 & 1 \end{bmatrix} \text{ X } 1/16$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-5-1-1-1, “Laplacian filter”.



<Lowpass> button

- Click the <Lowpass> button.

2-5-1-3 Image Sharpening

1 Sharpen filter

The sharpen filter turns blurred image into a clear image. The filter format is as shown below.

$$\begin{bmatrix} 0 & -1 & 0 \\ -1 & 5 & -1 \\ 0 & -1 & 0 \end{bmatrix}$$

The operation method is identical to Laplacian filtering except for the following point. See section 2-5-1-1-1, “Laplacian filter”.



<Sharpen> button

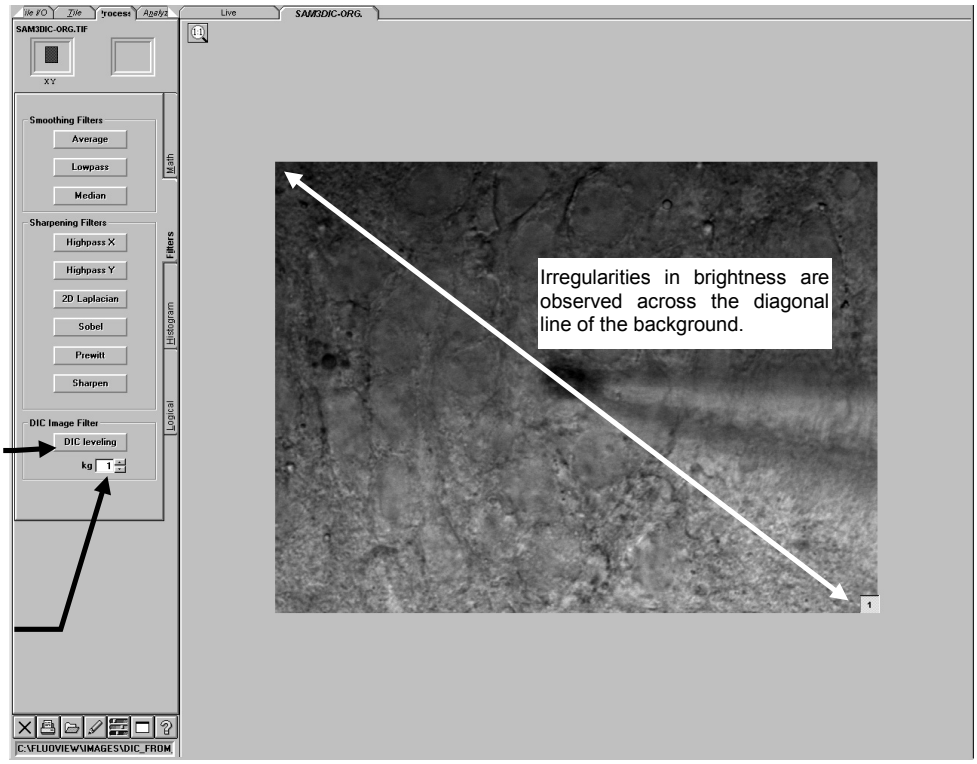
- Click the <Sharpen> button.

2-5-1-4 DIC Correcting DIC Level Irregularities

DIC level irregularities refers to uneven brightness of the image background which may be observed when a transmitted image is acquired in IDC observation.

The DIC level irregularities can be corrected to make the image easier to view.

1. Display the [Filters] sub-panel in the [Process] panel.



<DIC leveling>button

Automatic correction of irregularities of the image background

[kg] text box

Enter the correction factor of the image background.

Fig 2-73 [Filters] Sub-panel

2. Enter the appropriate correction factor for the image background in the [kg] text box.

TIP

The correction factor can be set between 0.1 and 1 in steps of 0.1.

The standard factor is 1. If the effect is extreme, try using 0.5.

3. Click the <DIC leveling> button.

   The [Level] panel is newly created in the [Display panel] and shows an image after the correction of the DIC level irregularities of the background.

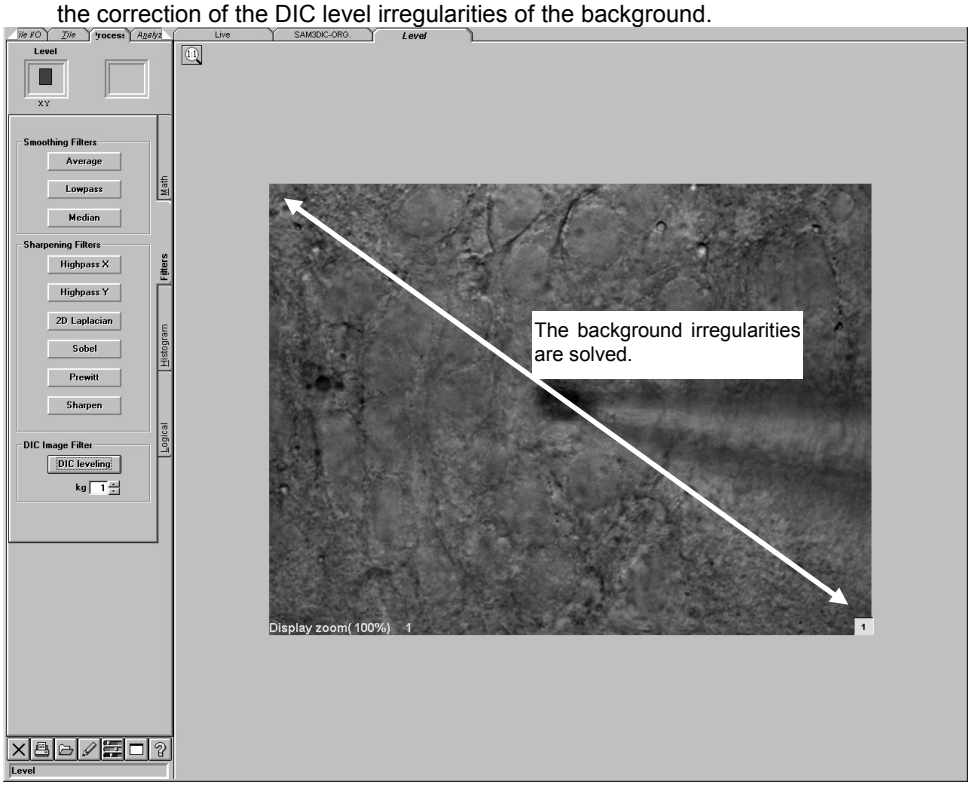


Fig. 2-74 [Level] Panel After DIC Level Correction

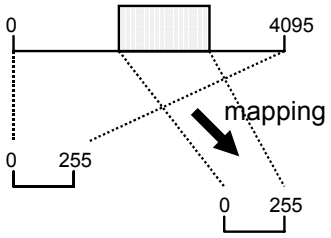
4. If the DIC level irregularities cannot be reduced, repeat steps 1 to 3 above by varying the correction factor.

2-5-2 Contrast Conversion

The LUT intensity can be mapped (re-assigned) while observing a histogram.

Mapping (re-assignment) results in changing the image contrast.

An image acquired by observation contains intensity information in values from 0 to 4095, but the intensity information used in actual display takes values from 0 to 255 by assigning the original values from 0 to 4095 to values from 0 to 255 usually. This facility changes the contrast by noticing a certain section between 0 and 4095 and mapping this section to values between 0 and 255.



1. Display the [Process] panel at the front.

2. Display the [Histogram] sub-panel of the [Process] panel at the front.

Sets the image to be subjected to contrast change. The icon of the image is displayed here.

[Channel] option button

Select the channel to be subjected to LUT intensity mapping (re-assignment).

<Compute Histogram> button

Displays the histogram.

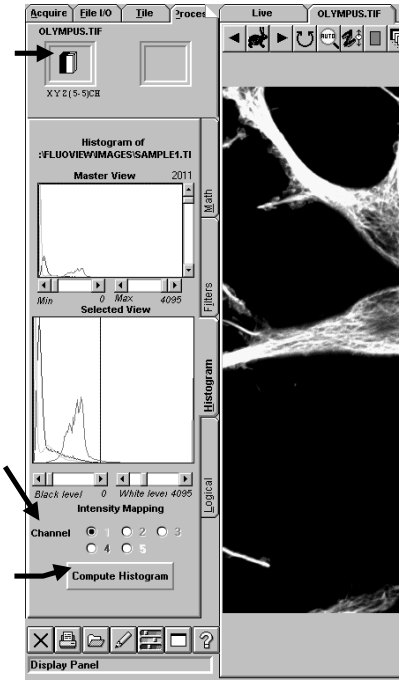


Fig. 2-75 [Histogram] Sub-panel

3. Display the [Display] panel of the image you want to change the contrast.

   The icon of the image is displayed in the frame at the top left of the [Process] panel



<Display channel switch> buttons

4. When the image was acquired in the multi-channel mode, select whether the LUT intensity mapping (re-assignment) is applied to multiple channels simultaneously or to a single channel.

   To select the target channel(s), use the <Display channel switch> buttons. The histogram of the selected channel(s) is displayed.

   Example) When only the Ch1 image is displayed, the histogram of Ch1 is displayed and mapping of only the Ch1 image is possible.

TIP

For the switching of channels, see section 2-4-3, “Switching the Display Channels”.

5. Click the <Compute Histogram> button. A histogram appears as shown below.

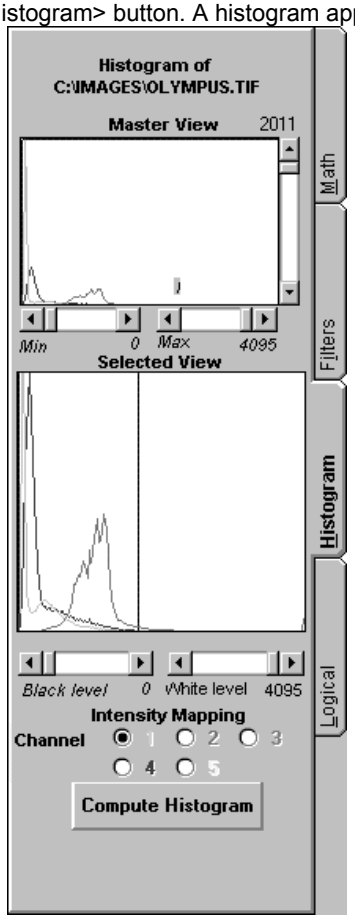


Fig. 2-76 Histogram

6. Enclose the histogram section of interest using the scale in the [Master View] field.

7. The magnified view of the region selected by the scale is shown in the [Selected View] field.

The vertical chart axis represents the degree and the horizontal axis represents the mapped intensity values.

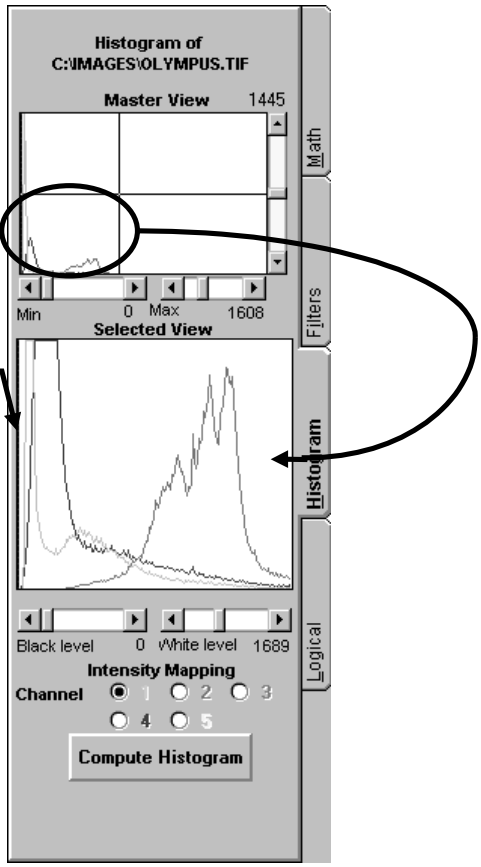


Fig. 2-77 Histogram of the Selected Region

8. When the image was acquired in the multi-channel mode, select the channel(s) to be subjected to LUT intensity mapping (re-assignment) using the [Channel] option buttons.

9. Select the region to be subjected to mapping (re-assignment) using the scale in the [Selected View] field. While moving the scale, confirm the change in contrast in the [Display] panel. The intensity values in the selected region are mapped (re-assigned) to intensity values from 0 to 255 and displayed.

2-5-3 Mathematical Operations Between Images

Arithmetic or logical operations can be applied between two different images or between an image and a constant.

2-5-3-1 Image Addition

Addition of an image to an image (constant to an image) is possible as described below.

1. Display the [Math] sub-panel of the [Process] panel.

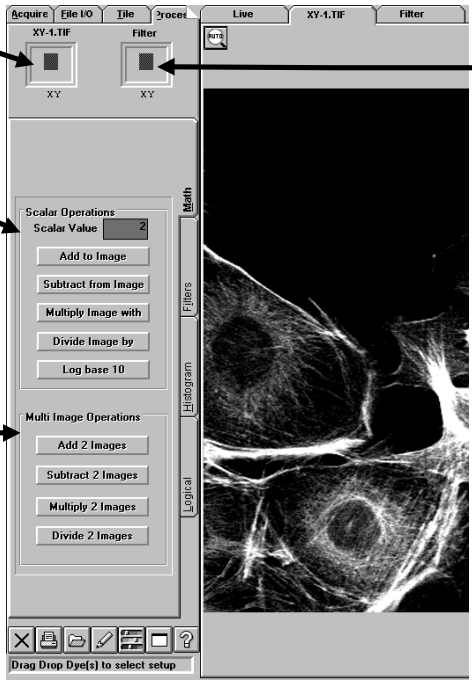
Sets the first image of the operation. The icon of the image is displayed here.

[Scalar Operations] group box

Provides the command buttons for use in operations between image and constant.

[Multi Image Operations] group box

Provides the command buttons for use in operations between two different images.



Sets the second image of the operation. The icon of the image is displayed here. This image needs not be set in operations between image and constant.

Fig. 2-78 [Math] Sub-panel

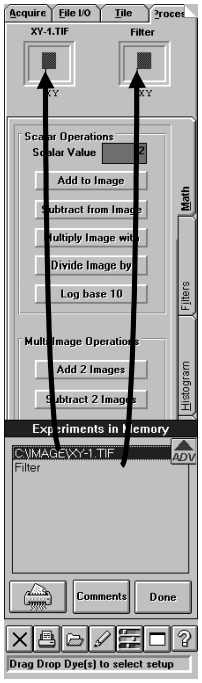


<Experiment List> button

2. Click the <Experiment List> button in the toolbar at the bottom of the [Process] panel. The [Experiments in Memory] dialog box appears as shown below.



Fig. 2-79 [Experiments in Memory] Dialog Box



3. From the [Experiments in Memory] dialog box, select the file name of the first image and drag it to the frame at the top left of the [Process] panel. The icon of the image is displayed in the frame at the top left of the [Process] panel.

TIP

Before the dragging and dropping, the frame at the top left shows the icon of the image file displayed in the Display panel.

TIP

The mouse pointer turns into the image icon during dragging.

4. From the [Experiments in Memory] dialog box, select the file name of the second file and drag it to the frame at the top right of the [Process] panel. The icon of the image is displayed in the frame at the top right of the [Process] panel.

(No second file dragging is needed when the image processing is by value.)

TIP

The mouse pointer turns into the image icon during dragging.

5. Click the <Done> button in the [Experiments in Memory] dialog box to close it.

6. Enter the constant for use in operation in the [Scalar Value] text box in the [Scalar Operations] group box.
   (This step is required only for operation between an image and a constant.)



<Add 2 Images> button

7. **To add an image to an image:**
   Click the <Add 2 Images> button in the [Multi Image Operations] group box. A new [Display] panel showing [Image+Image] in the page tab appears, displaying the image obtained by the addition operation.



<Add to Image> button

   To add a constant to an image:
   Click the <Add to Image> button in the [Scalar Operations] dialog box. A new [Display] panel showing [Image+Const] in the page tab appears, displaying the image obtained by the addition operation.

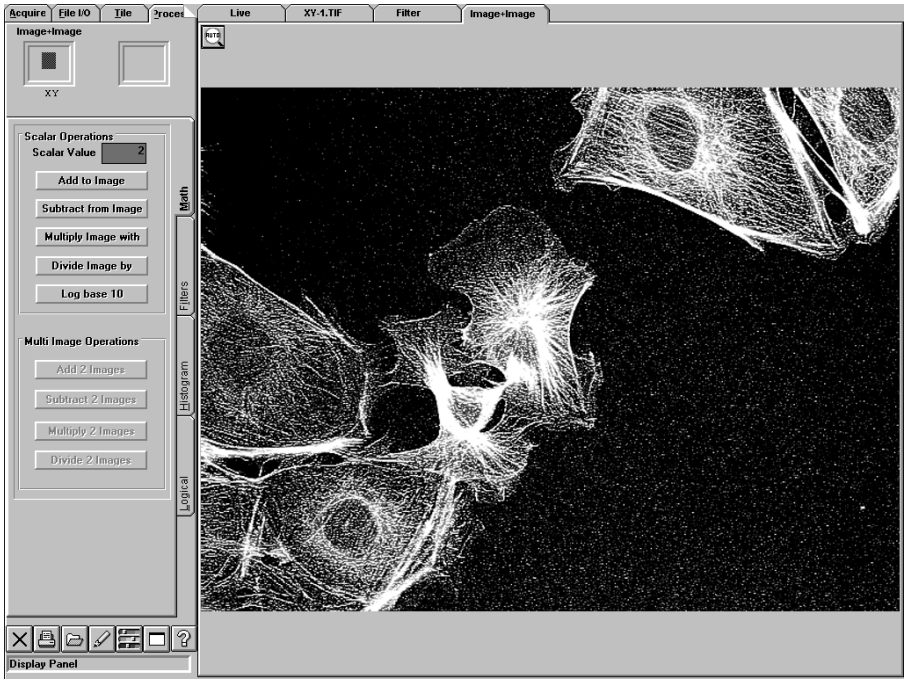


Fig. 2-80 [Image+Image] Panel

2-5-3-2 Image Subtraction

Subtraction of an image from an image (constant from an image) is possible as described below.

The operation method is identical to Image + Image (Image + Constant) except for the following point. See section 2-5-3-1, “Image Addtion”.



<Subtract 2 Images> button

- **To subtract an image from an image:**

  Click the <Subtract 2 Images> button in the [Multi Image Operations] group box. A new [Display] panel showing [Image-Image] in the page tab appears, displaying the image obtained by the subtraction operation.



<Subtract from Image> button

To subtract a constant from an image:

Click the <Subtract from Image> button in the [Scalar Operations] group box. A new [Display] panel showing [Image-Const] in the page tab appears, displaying the image obtained by the subtraction operation.

2-5-3-3 Image Multiplication

Multiplication of an image by an image (image by a constant) is possible as described below.

The operation method is identical to Image + Image (Image + Constant) except for the following point. See section 2-5-3-1, “Image Addition”.



<Multiply 2 Images> buttotn

- **To multiply an image by an image:**

  Click the <Multiply 2 Images> button in the [Multi Image Operations] group box. A new [Display] panel showing [Image*Image] in the page tab appears, displaying the image obtained by the multiplication operation.



<Multiply Image with> button

To multiply an image by a constant:

Click the <Multiply Image with> button in the [Scalar Operations] group box. A new [Display] panel showing [Image*Const] in the page tab appears, displaying the image obtained by the multiplication operation.

2-5-3-4 Image Division

Division of an image by an image (image by a constant) is possible as described below.

The operation method is identical to Image + Image (Image + Constant) except for the following point. See section 2-5-3-1, “Image Addition”.



<Divide 2 Images> button

- **To divide an image by an image:**

  Click the <Divide 2 Images> button in the [Multi Image Operations] group box. A new [Display] panel showing [Image/Image] in the page tab appears, displaying the image obtained by the division operation.



<Divide Image by> button

To divide an image by a constant:

Click the <Divide Image by> button in the [Scalar Operations] group box. A new [Display] panel showing [Image/Const] in the page tab appears, displaying the image obtained by the division operation.

2-5-3-5 NOT Image

The NOT operation of an image allows the bright and dark areas of the image to be reversed.

1. Display the [Logical] sub-panel of the [Process] panel at the front.

Sets the image to be subjected to operation. The icon of the image is displayed here.

<NOT Image> button
Executes a NOT operation.

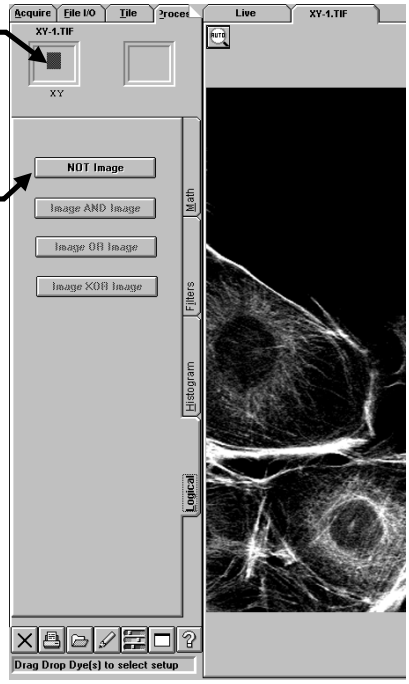


Fig. 2-81 [Logical] Sub-panel

2. Display the [Display] panel of the image to be subjected to NOT operation at the front. The icon of the image is displayed in the frame at the top left of the [Process] panel.



<NOT Image> button

3. Click the <NOT Image> button. A new [Display] panel showing [ImageNot] in the page tab appears, showing the image obtained by the operation.

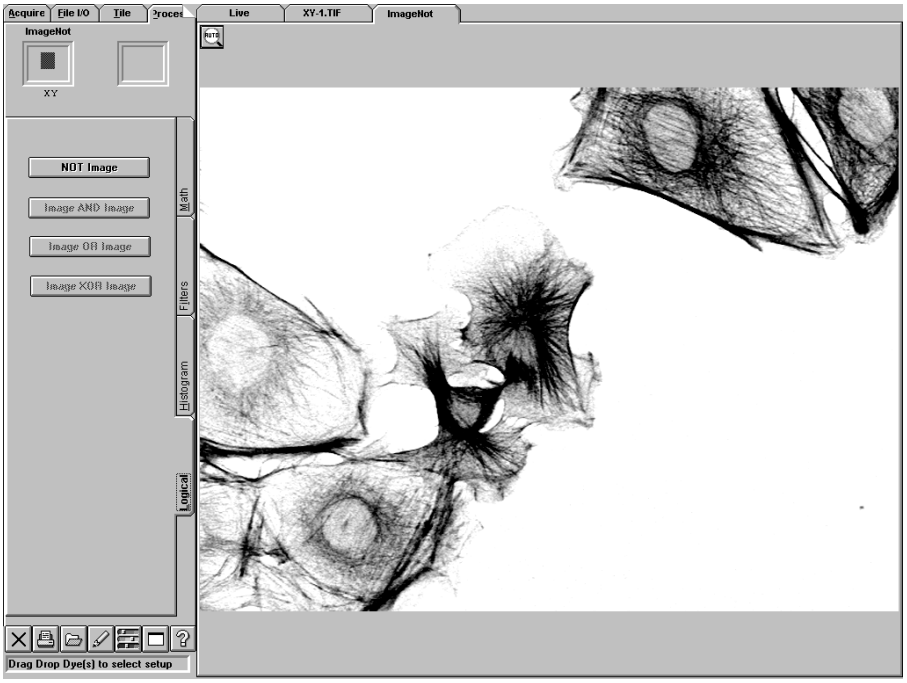


Fig. 2-82 [ImageNot] Panel

2-5-3-6 Image AND Image

Two different images can be ANDed.

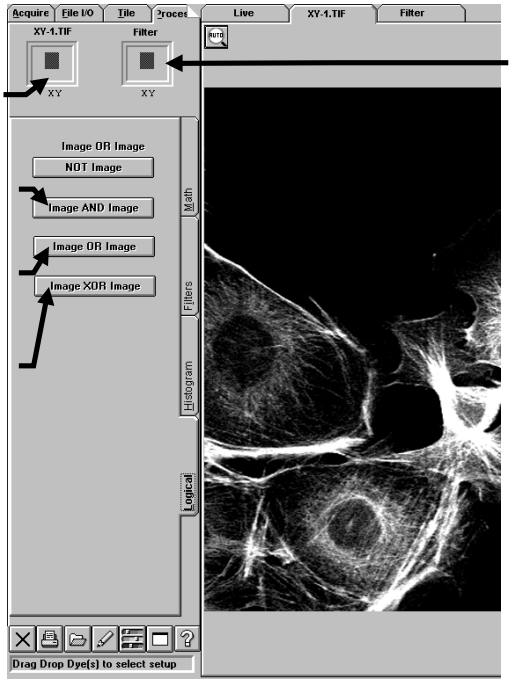
1. Display the [Logical] sub-panel of the [Process] panel at the front.

Sets the image to be subjected to operation. The icon of the image is displayed here.

<Image AND Image> button
Executes an AND operation.

<Image OR Image> button
Executes an OR operation.

<Image XOR Image> button
Executes an exclusive OR operation.



Sets the second image of the operation. The icon of the image is displayed here.

Fig. 2-83 [Logical] Sub-panel



<Experiment List> button

2. Click the <Experiment List> button in the toolbar at the bottom of the [Process] panel. The [Experiments in Memory] dialog box appears as shown below.

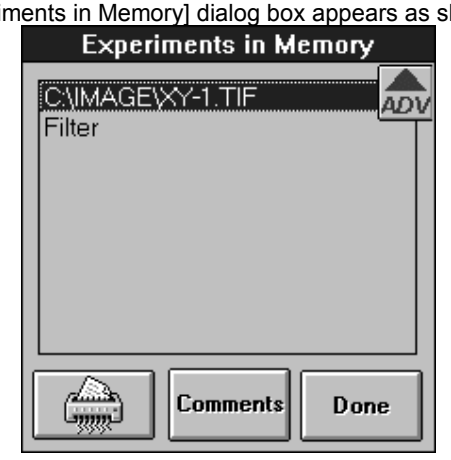
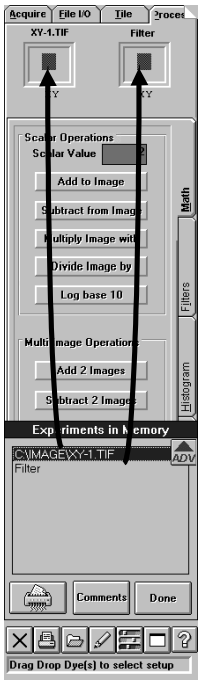


Fig. 2-84 [Experiments in Memory] Dialog Box



3. From the [Experiments in Memory] dialog box, select the file name of the first image and drag it to the frame at the top left of the [Process] panel. The icon of the image is displayed in the frame at the top left of the [Process] panel.

TIP Before the dragging and dropping, the frame at the top left shows the icon of the image file displayed in the [Display] panel.

TIP The mouse pointer turns into the image icon during dragging.

4. From the [Experiments in Memory] dialog box, select the file name of the second file and drag it to the frame at the top right of the [Process] panel. The icon of the image is displayed in the frame at the top right of the [Process] panel.

TIP The mouse pointer turns into the image icon during dragging.

5. Click the <Done> button in the [Experiments in Memory] dialog box to close it.



<Image AND Image> button

6. Click the <Image AND Image> button. A new [Display] panel showing [Image AND Image] in the page tab appears, showing the image obtained by the operation.



Fig. 2-85 [Image AND Image] Panel

2-5-3-7 Image OR Image

Two different images can be ORed.

The operation method is identical to the AND operation between two different images except for the following point. See section 2-5-3-6, “Image AND Image”.



<Image OR Image> button

- Click the <Image OR Image> button. A new [Display] panel showing [Image OR Image] in the page tab appears, showing the image obtained by the operation.

2-5-3-8 Image XOR Image

Two different images can be XORed.

The operation method is identical to the AND operation between two different images except for the following point. See section 2-5-3-6, “Image AND Image”.



<Image XOR Image> button

- Click the <Image XOR Image> button. A new [Display] panel showing [Image XOR Image] in the page tab appears, showing the image obtained by the operation.

2-6 Image Analysis

Images can be analyzed using the [Analyze] panel. Display the [Analyze] panel at the front.

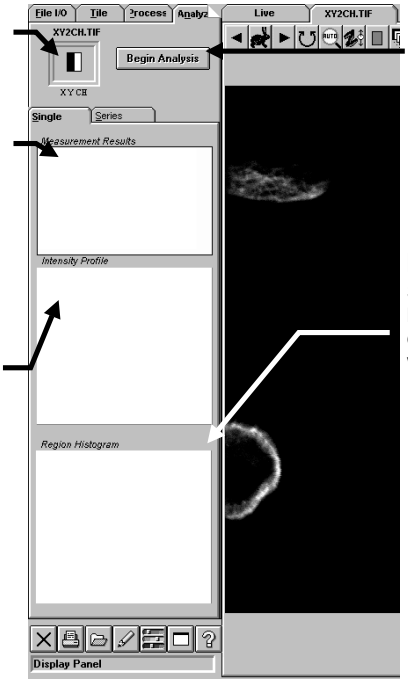
Displays the icon of the image being displayed (image to be subjected to analysis).

[Measurement Results] box

Shows the measurement data of the specified line or region.

[Intensity Profile] box

Shows the intensity profile chart of the specified line or region. When a line is specified, the line profile is displayed, and double-clicking this field displays the [Enhanced Profile Plot] window. When a region is specified, the bird's eye view is displayed, and double-clicking this field displays the [Intensity Map] window.



<Begin Analysis> button

Starts analysis.

[Region Histogram] box

Shows the histogram of the specified line or region. Double-clicking this field displays the [Enhanced Histogram Plot] window.

Fig. 2-86 [Analyze] Panel

2-6-1 Checking the Intensity of a Specific Part

2-6-1-1 Intensity Values on a Line (Line Profile)

The intensity values on a line in an image can be displayed graphically.

1. Display the [Single] sub-panel at the front.
2. Display the [Display] panel of the image to be subjected to the intensity checking at the front.
3. Click the <Annotate> button in the toolbar at the bottom of the [Analyze] panel. A list of buttons appears as shown below.


<Annotate> button



4. From the displayed buttons, click the <Line> button, <Poly Line> button or <Free Line> button.
5. Specify the straight line, polygonal line or free line on the image in the [Display] panel.

   They can be specified as described below.


<Line> button

- To specify a straight line:

  On the image, place the mouse pointer on the point you want to start the straight line and drag until the point you want to end it.


<Poly Line> button

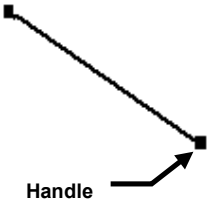
- To specify a polygonal line:

  On the image, click the points corresponding to the start point, peak points and end point of the desired polygonal line, then click the right button of the mouse to set the specification.



<Free Line>button

- To specify a free line:
  On the image, drag the mouse pointer along the line to be checked.
  The line is displayed on the image together with the handles on it. The intensity profile can be displayed while the handles are displayed.



NOTE

When the moues is clicked in other place than on the specified line, the handles on the line disappear. The intensity profile cannot be displayed when the handles are not displayed.

TIP

The checked line can be moved, deleted or changed of size or color. This is possible with the same method as entering comment in the image.
For details, see sections 2-11-6, 2-11-7, 2-11-8 and 2-11-9 in section 2-11, “Entering Comment in Image”.

6. Click the <Annotate> button so that the list of buttons disappears.
7. Click the <Begin Analysis> button. The intensity profile of the specified line will be displayed in the [Intensity Profile] box of the [Analyze] panel.



<Annotate> button

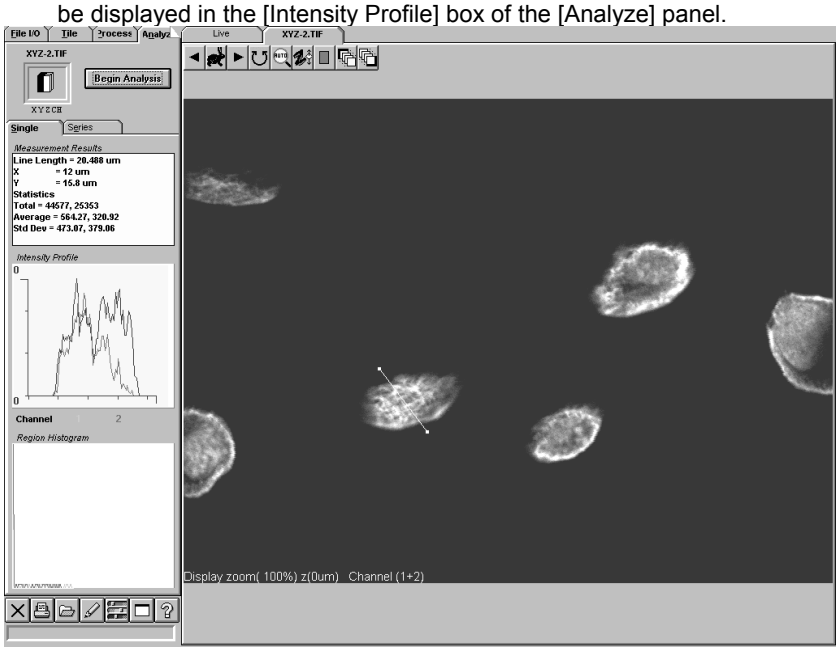


Fig. 2-87 Panel After Analysis (Line Specification)

8. Double-click the [Intensity Profile] button. The [Enhanced Profile] window appears as shown below.

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-14, "Changing the Chart Display Method" for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

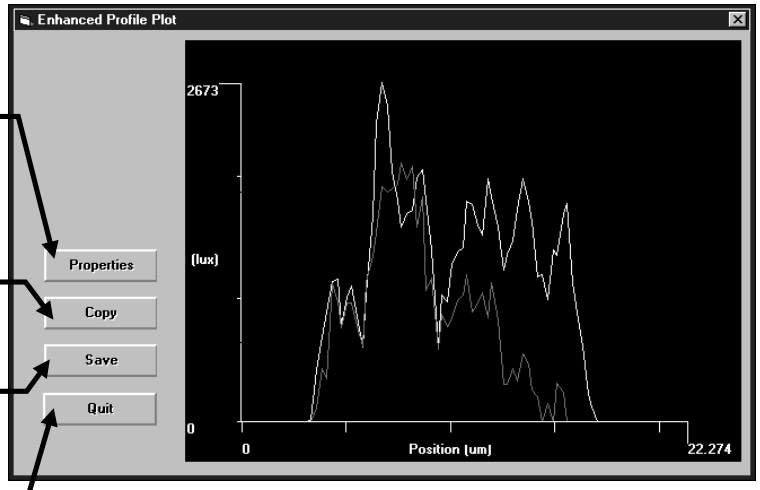


Fig. 2-88 [Enhanced Profile Plot] Window

TIP When a desired area is specified by dragging the left button of the mouse on the graph, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP When the mouse pointer is placed on a graph line while the Ctrl or Alt key is held depressed, the coordinates can be displayed.

TIP The displayed data can be applied to other applications.

See section 2-10, "Transferring Data to Another Application" for details.

2-6-1-2 Intensity Values on a Planar Region (Bird's Eye View)

The intensity values on a region in an image can be displayed graphically.

1. Display the [Single] sub-panel at the front.

2. Display the [Display] panel of the image to be subjected to the intensity checking at the front.

3. When the image was acquired in the multi-channel mode, select whether the multiple channels are analyzed simultaneously or only one channel is analyzed.
   To select the target channel(s), use the <Display channel switch> buttons. Only the channel(s) being displayed will be analyzed.
   Example) When only the Ch1 image is displayed, only the Ch1 image is analyzed.



<Display channel switch> button

TIP For the switching of channels, see section 2-4-3, "Switching the Display Channels".


<Annotate> button

4. Click the <Annotate> button in the toolbar at the bottom of the [Analyze] panel. A list of buttons appear as shown below.



5. From the displayed buttons, click the <Rectangular> button, <Circle> button or <Polyregion> button.

6. Specify the region to be checked in the image in the [Display] panel.
   They can be specified as described below.


<Rectangular> button

- To specify a rectangle:
  On the image, drag the mouse pointer along the diagonal line of the desired rectangle, from the top left corner to the bottom right corner.


<Circle> button

- To specify a circle or ellipse:
  On the image, assume a rectangle circumscribing the circle to be checked and drag the mouse pointer along the diagonal line between opposite corners of the rectangle.

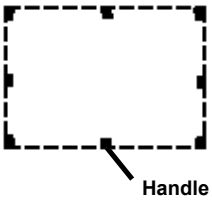

<Polyregion> button

- To specify a polygonal region:
  On the image, click the points corresponding to the corners of the polygon to be checked. After clicking the last corner point, click the right button of the mouse to connect the last clicked point to the first clicked point.


<Free Region> button

- To specify a free region:
  On the image, specify a region by dragging. Then release the mouse button to complete dragging. The point where the dragging was ended will be connected to the point where it was started.

A region is displayed on the image together with handles on the perimeter. The region is selected as the target of the bird's eye view while the handles are displayed.



NOTE

If the mouse is clicked in other place than inside the region specified on the image, the handles will disappear. The bird's eye view cannot be displayed while the handles are not displayed.

TIP

The checked region can be moved, deleted or changed of size or color.

This is possible with the same method as entering comment in the image.

For details, see sections 2-11-6, 2-11-7, 2-11-8 and 2-11-9 in section 2-11, “Entering Comment in Image”.



<Annotate> button

7. Click the <Annotate> button so that the list of buttons disappears.
8. Click the <Begin Analysis> button. The bird’s eye view of the specified region will be displayed in the [Intensity Profile] box of the [Analyze] panel.

TIP

The [Measurement Results] box in the [Single] sub-panel shows the measurement results such as the area (Area), horizontal and vertical lengths (X/Y, Z or T) and perimeter length (Perimeter) of the region, and the statistic result of each channel such as the total (Total), average (Average) and standard deviation (Std Dev).

9. When the image was acquired in the multi-channel mode, the channel(s) to be subjected to the bird's eye view display can be selected using the [Channel] option buttons.

Shows:
Measurement results including;
· Perimeter
· Area
· X/Y, Z or T
Statistical channel data including;
· Total
· Average
· Std Dev

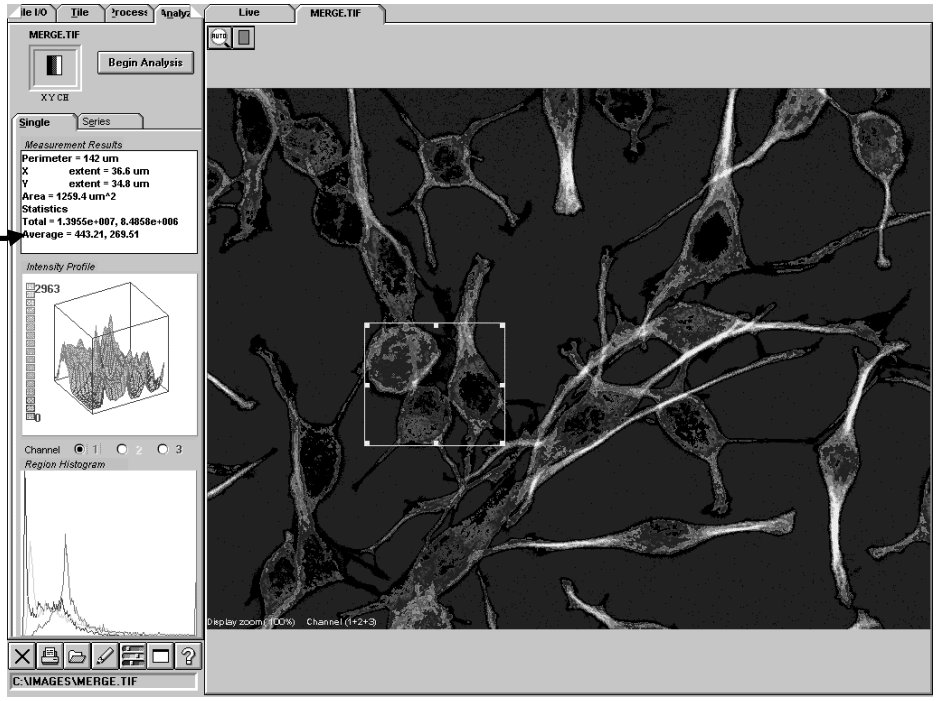


Fig. 2-89 Panel After Analysis (Region Specification)

10. Double-click the [Intensity Profile] button. The [Intensity Map] window appears as shown below.

[Angle] scale

Sets the angle in the horizontal direction.
The result can be confirmed with the small bird's eye view in the frame on the

[Tilt] scale

Sets the angle in the vertical direction. The result can be confirmed with the small bird's eye view in the frame on the top left.

<Plot> button

Displays the bird's eye view with the angles set above.

<Spin> button

Spins the bird's eye view by one turn. The spinning starts from the front.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze]

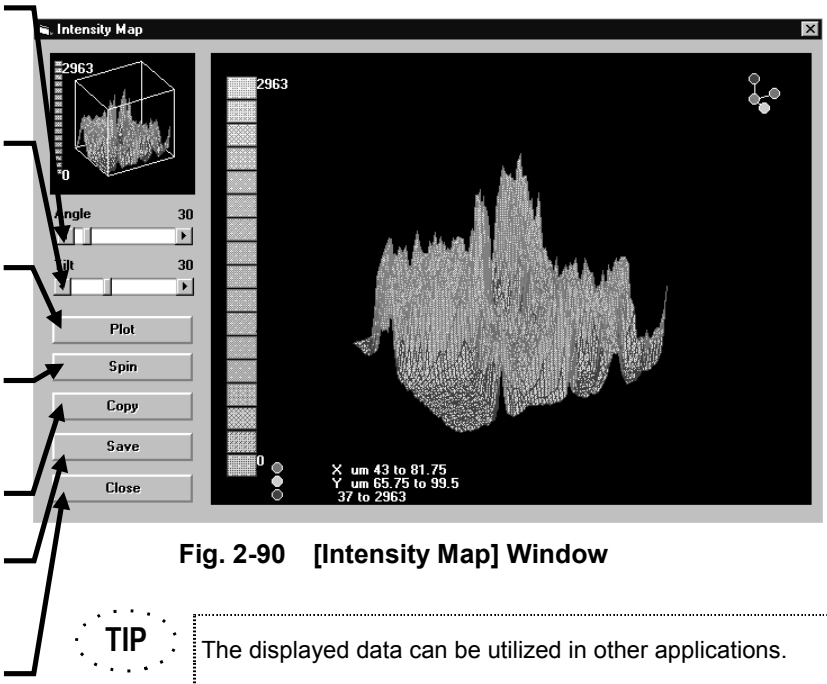


Fig. 2-90 [Intensity Map] Window

TIP

The displayed data can be utilized in other applications.

See section 2-10, "Transferring Data to Another Application" for details.

2-6-2 Checking the Intensity Distribution of a Specific Part

2-6-2-1 Intensity Distribution on a Line (Histogram)

The histogram on a line in an image can be displayed.

The histogram is displayed in the [Region Histogram] box in the [Single] sub-panel.

The operation method is identical to displaying the intensity profile on a line. See section 2-6-1-1, “Intensity Values on a Line (Line Profile)”.

Double-click the [Region Histogram] window. The [Enhanced Histogram Plot] windows appears as shown below.

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-14, “Changing the Chart Display Method” for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

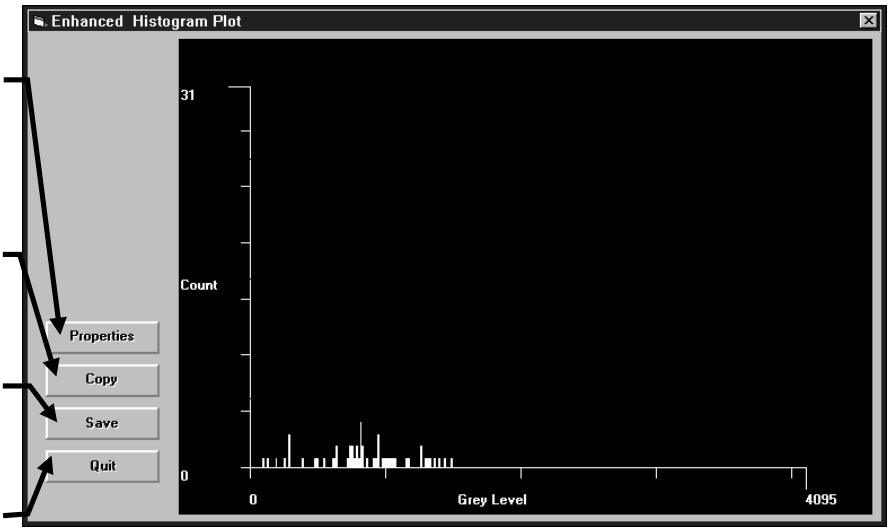


Fig. 2-91 [Enhanced Histogram Plot] Window (Line Specification)

TIP When a desired area is specified by dragging the left button of the mouse on the graph, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP When the mouse pointer is placed on a graph line while the Ctrl or Alt key is held depressed, the coordinates can be displayed.

TIP The displayed data can be applied to other applications.
See section 2-10, “Transferring Data to Another Application” for details.

2-6-2-2 Intensity Distribution on a Planar Region (Histogram)

The histogram on a region in an image can be displayed.

The histogram on a region in an image can be displayed.

The operation method is identical to displaying the intensity profile on a line. See section 2-6-1-1, “Intensity Values on a Line (Line Profile)”.

Double-click the [Region Histogram] window. The [Enhanced Histogram Plot] windows appears as shown below.

<Properties> button
Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.
See section 2-14, “Changing the Chart Display Method” for details.

<Copy> button
Copies the plotted image in the clipboard.

<Save> button
Saves the profile data in a file using an Excel-compatible format.

<Close> button
Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

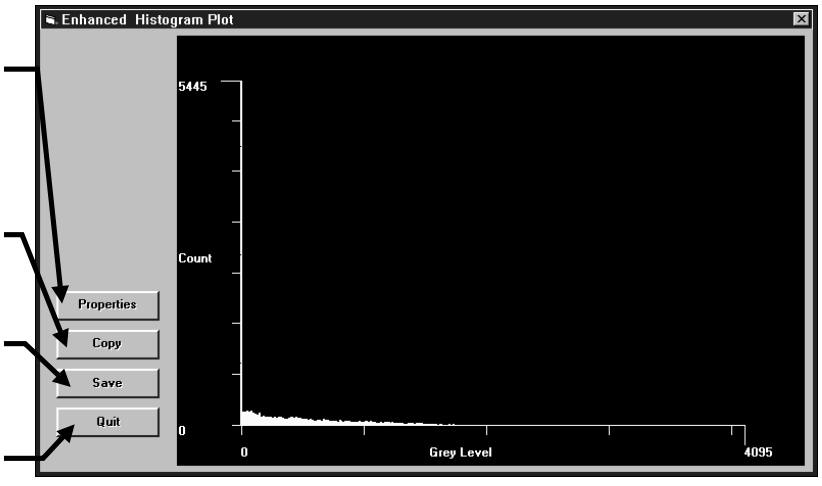


Fig. 2-92 [Enhanced Histogram Plot] Window (Line Specification)

TIP When a desired area is specified by dragging the left button of the mouse on the graph, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP When the mouse pointer is placed on a graph line while the Ctrl or Alt key is held depressed, the coordinates can be displayed.

TIP The displayed data can be applied to other applications.
See section 2-10, “Transferring Data to Another Application” for details.

2-6-3 Measuring a Part

2-6-3-1 Length Measurement

The length between two points in an image or the perimeter of a region in an image can be measured.

The [Measurement Results] box in the [Single] sub-panel shows the measurement results such as the length between 2 points (Length) or perimeter of the region (Perimeter) and the horizontal and vertical lengths (X/Y, Z or T) of the region, and the statistic result of each channel such as the total (Total), average (Average) and standard deviation (Std Dev).

The operation method for measuring the length between 2 points is identical to that for displaying the intensity profile of a line. See section 2-6-1-1, “Intensity Values on a Line (Line Profile)”. The operation method for measuring the perimeter of a region is identical to that for displaying the bird’s eye view of a region. See section 2-6-1-2, “Intensity Values on a Planar Region (Bird’s Eye View)”.

TIP

The measurement results can be written in comment by copying and pasting them in the [Image Comments] dialog box in the [Experiments in Memory] dialog box.
See section 2-3-4, “Saving Comment Together with Image” for details.

2-6-3-2 Area Measurement

The area of a region in the image can be measured.

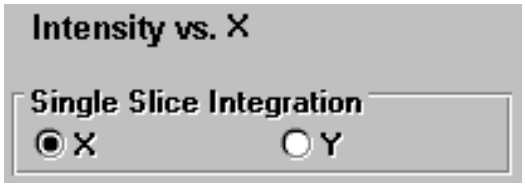
The [Measurement Results] box in the [Single] sub-panel shows the measurement results such as the area of the region (Area), perimeter of the region (Perimeter) and the horizontal and vertical lengths (X/Y, Z or T) of the region, and the statistic result of each channel such as the total (Total), average (Average) and standard deviation (Std Dev).

The operation method is identical to that for displaying the bird’s eye view of a region. See section 2-6-1-2, “Intensity Values on a Planar Region (Bird’s Eye View)”.

2-6-3-3 Measuring the Change in Mean Value of Intensity

The mean value of the intensity in a region specified in an image can be measured and displayed graphically.

1. Display the [Series] sub-panel at the front.
2. Display the [Display] panel of the image that you want to check the intensity at the front.
3. When the image is composed of multiple image slices, operations will be applied to each slice of the image. When the image is composed of a single image slice or when one of the multiple image slices is selected, the [Single Slice Integration] group box appears as shown below. Select the direction of interest (X or Y) using the option buttons.

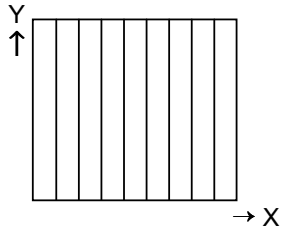


NOTE

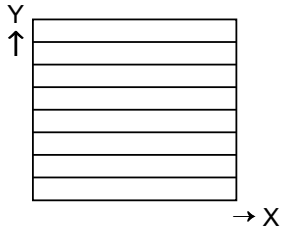
When the selected image was acquired in the XY observation mode, the [Single Slice Integration] group box shows the option buttons for selection of the X- or Y-direction. When the selected image was acquired in the XT observation mode, the option buttons for selection of the X- or T-direction are displayed.

Example) When the direction of interest is selected in the [Single Slice Integration] group box, line-by-line computation operation starts on the perpendicular lines to the selected direction. For example, an image acquired in XY observation is checked as shown below.

(When the X-direction is selected)



(When the X-direction is selected)




<Set start position> button


<Set end position> button


<Display>button


<XYZ series> button


<XYT series> button



<Display channel switch> buttons

TIP

When the image is composed of multiple image slices, the range of image slices to be subjected to the operation can be set using the <Set start position> and <set end position> buttons above the images. First display the image slice to start the operation using the <Display> button and click the <Set start position> button. Then, set the image slice to end the operation in the same way as above.

TIP

With an image acquired in XYZT observation, the slice images (cross-section (Z)/time lapse (T)) to be subjected to the operation can be selected using the <XYZ series> and <XYT series> buttons above the image.

4. When the image was acquired in the multi-channel mode, select whether the multiple channels are operated simultaneously or only one channel is operated. To select the target channel(s), use the <Display channel switch> buttons. Only the channel(s) being displayed will be analyzed.
   Example) When only the Ch1 image is displayed, only the Ch1 image is analyzed.

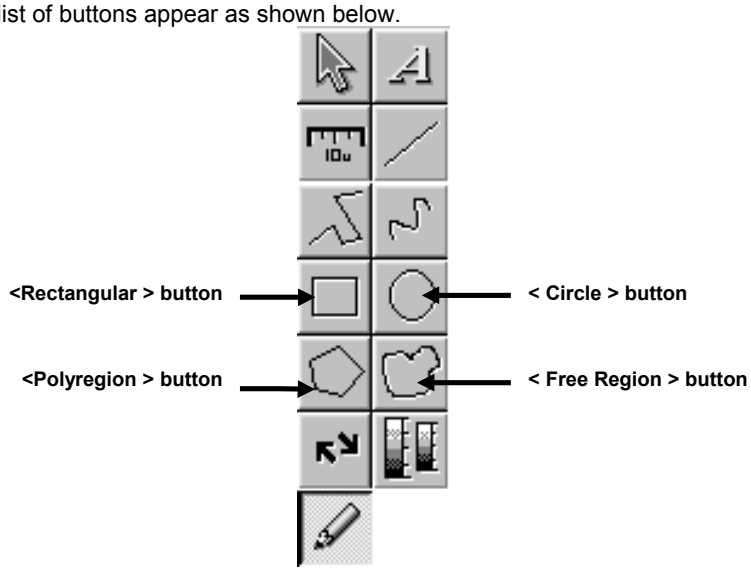
TIP

For the switching of channels, see section 2-4-3, “Switching the Display Channels”.



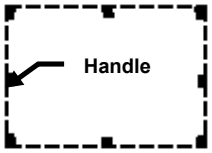
<Annotate> button

5. Click the <Annotate> button in the toolbar at the bottom of the [Analyze] panel. A list of buttons appear as shown below.



6. From the displayed buttons, click the <Rectangular> button, <Circle> button, <Polyregion> button or <Free Region> button.

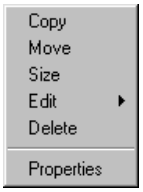
7. Specify the region to be checked in the image in the [Display] panel.
   For the specification method, see section 2-6-1-2, “Intensity Values on a Planar Region (Bird’s Eye View)”.
   A region is displayed on the image together with handles on the perimeter. The region is selected as the target of mean value computation operation while the handles are displayed.



NOTE

If the mouse is clicked in other place than inside the region specified on the image, the handles will disappear. On the contrary, clicking the mouse in a region displays handles around it. The operation cannot be executed while the handles are not displayed.

8. Click the mouse. A pop-up menu as shown below appears. Select [Properties] from the menu.



9. The [Properties] dialog box as shown below appears. Display the [Color] panel at the front.

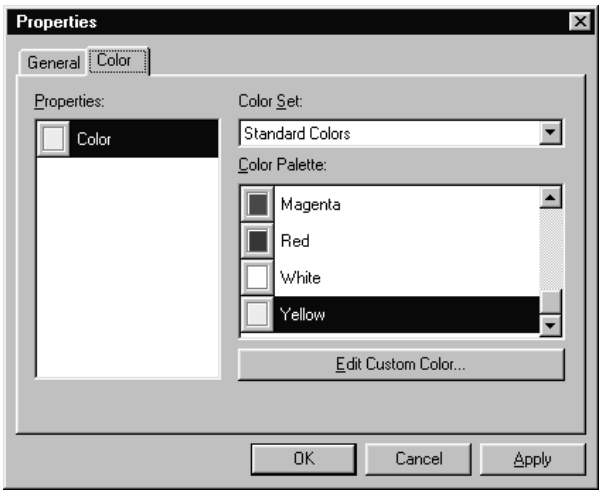


Fig 2-93 [Properties] Dialog Box

10. Select the desired color from the [Color Palette] list box.
11. It is also possible to specify more than one region simultaneously and display their operation results together. First specify the regions by repeating steps 7 and 8 above for each. Use different colors for the regions. After having set the regions, click the mouse in the first region. With the second regions and after, click the mouse while pressing the Shift key depressed.


<Annotate> button

12. Click the <Annotate> button so that the list of buttons disappears.
13. Using the scale in the [Threshold] group, set the threshold value for the intensity values used in operation. The intensity data above the threshold values set here will be used in the operation.

14. Click the <Begin Analysis> button. The mean value of the specified regions will be displayed graphically in the [Mean Intensity] box.

NOTE **The colors of the chart lines corresponding to the colors assigned to the regions.**

NOTE **When the image was acquired in the multi-channel mode, the channel number is displayed to the right of each chart line.**

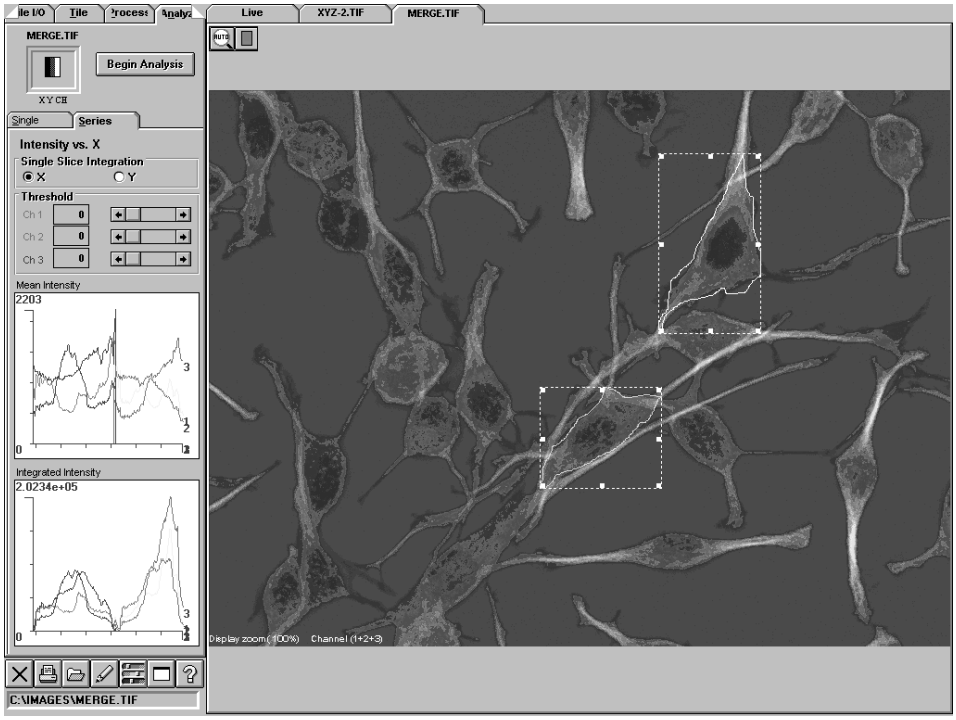


Fig. 2-94 Panel After Analysis

15. Double-click the [Mean Intensity] box. The [Average Intensity Trace] window appears as shown below

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-14, “Changing the Chart Display Method” for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Average Intensity Trace] window and returns to the [Analyze] panel.

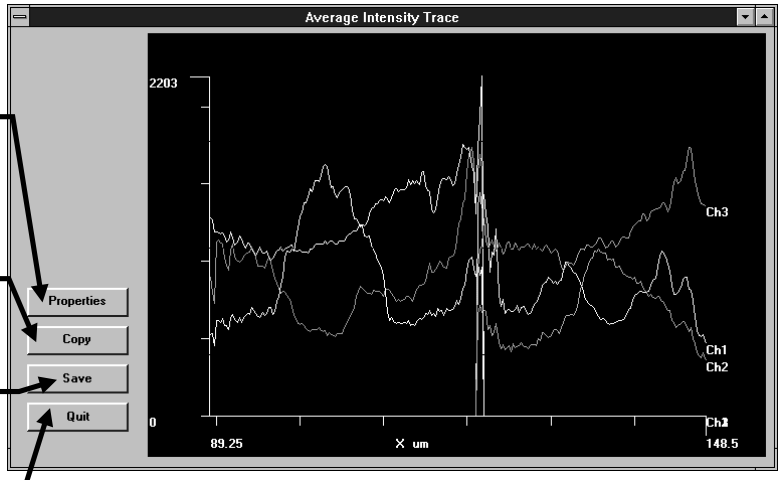


Fig. 2-95 [Average Intensity Trace] Window

TIP When a desired area is specified by dragging the left button of the mouse on the graph, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP When the mouse pointer is placed on a graph line while the Ctrl or Alt key is held depressed, the coordinates can be displayed.

TIP The displayed data can be applied to other applications.

See section 2-10, “Transferring Data to Another Application” for details.

2-6-3-4 Measuring the Change in Integrated Intensity

The total value of the intensity in a region specified in an image can be measured and displayed graphically.

The operation results are displayed graphically in the [Integrated Intensity] box in the [Series] sub-panel.

The operation method is completely identical to that for obtaining the mean value of intensity. See section 2-6-3-3, “Measuring the Change in Mean Value of Intensity”.

Double-click the [Integrated Intensity] box. The [Integrity Intensity Trace] window appears as shown below.

<Properties> button

Displays the [Editing] dialog box for use in detailed setting of the chart or change of the chart display.

See section 2-14, “Changing the Chart Display Method” for details.

<Copy> button

Copies the plotted image in the clipboard.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Integrated Intensity Trace] window and returns to the [Analyze] panel.

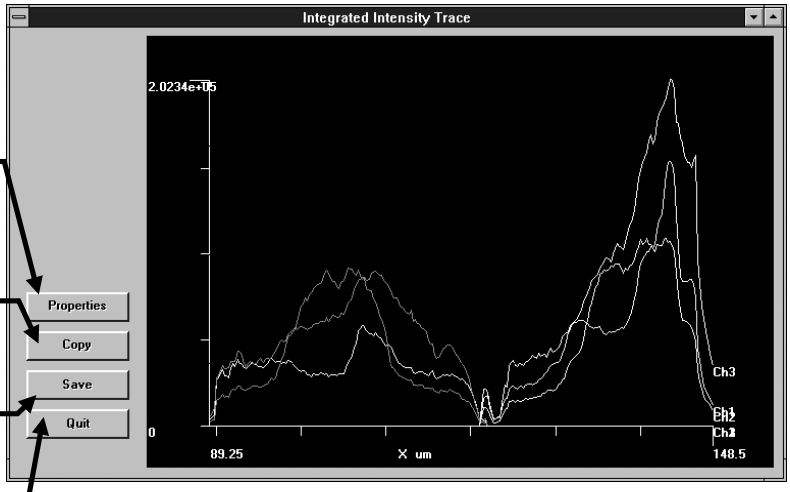


Fig. 2-96 [Integrated Intensity Trace] Window

TIP When a desired area is specified by dragging the left button of the mouse on the graph, the specified area can be magnified.

TIP When the right button of the mouse is dragged on the graph, the graph can be scrolled.

TIP

The magnification or scrolling of the graph can be canceled by dragging the left button of the mouse from the bottom left to the right of the magnified graph.

TIP

When the mouse pointer is placed on a graph line while the Ctrl or Alt key is held depressed, the coordinates can be displayed.

TIP

The displayed data can be applied to other applications.

See section 2-10, “Transferring Data to Another Application” for details.

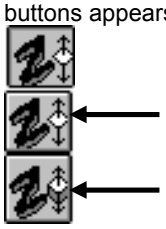
2-7 Building an Image from a Different Viewpoint

2-7-1 Building Extended Focus Image from XYZ Image

2-7-1-1 Display Switching to Built Image

An extended focus image can be built from XYZ (multiple sections) images and the display can be switched to show the built image.

1. Display the [Display] panel of the XYZ (multiple sections) image.
2. The following button is displayed at the top of the [Display] panel.
   (Usually, only the <XYZ series> button is displayed. When it is clicked, a list of buttons appears as shown below.)



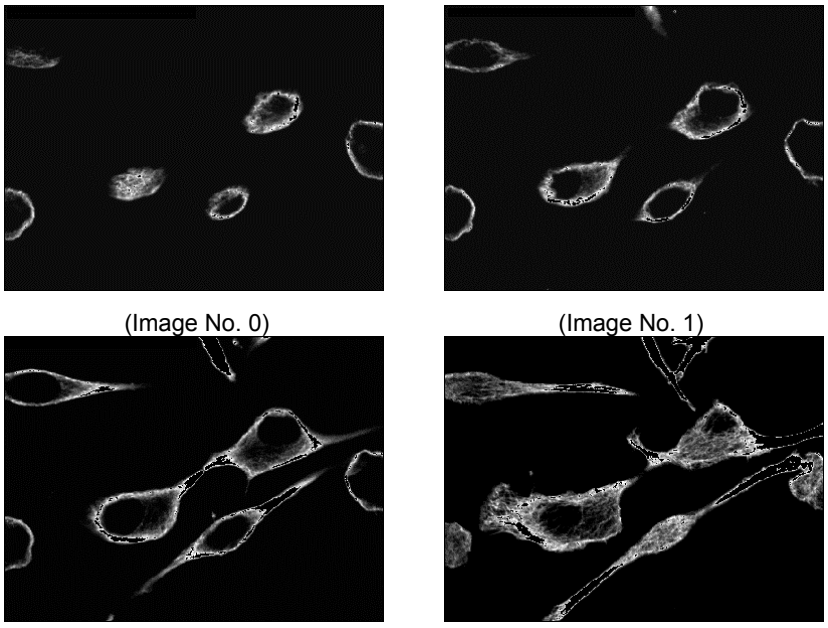
<XYZ series> button
Displays only one of multiple sections image slices.

<Extend> button
Displays the extend image.

3. Click the <Extend> button.
4. Click the <Display> button at the top of the [Display panel] repeatedly to build the extended-focus image.
   (The extended-focus image can also be displayed by clicking and holding the <display> button for successive display.)



<Display>button



(Image No. 0) (Image No. 1)

(Image No.2) (Image No. 3)

Fig. 2-97 Four Images Used in Building Extended-Focus Image

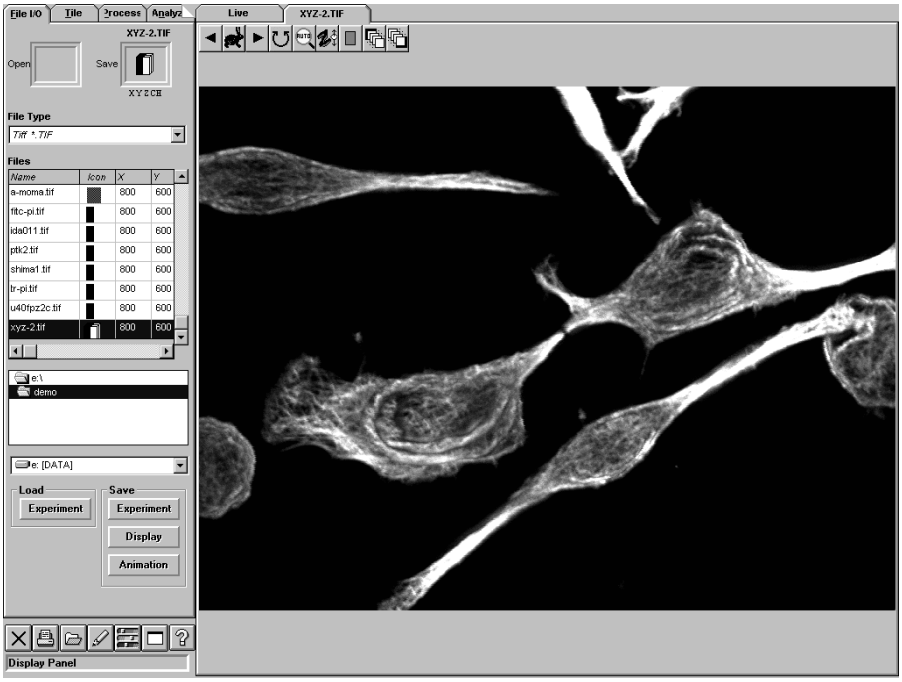


Fig. 2-98 Panel Showing an Extended-Focus Image


<Set start position> button


<Set end position> button

Among the multiple image slices composing the XYZ image, the range of image slices to be used in extend image building can be specified.

1. Display the image slice to be set as the start image by using the <Frame advance> or <Successive display> buttons at the top of the [Display] panel.
2. Click the <Set start position> button at the top of the [Display] panel.
3. Display the image slice to be set as the end image by using the <Frame advance> or <Successive display> buttons at the top of the [Display] panel.
4. Click the <Set end position> button at the top of the [Display] panel.

NOTE **The <Set start position> and <Set end position> buttons are valid in the pushed-in condition. To cancel a previously set start or end position, click the <Set start position> or <Set end position> button again.**

NOTE **When it is required to analyze the built extended-focus image or save it in the Fluoview Multi Tiff format, create the extended-focus image as a separate image from the original image. See section 2-7-1-2, "Turning Built Image into Single Image".**

2-7-1-2 Turning Built Image into Single Image

From XYZ (multiple sections) image, an extended-focus image can be built as a separate image from the original image.

Use the [Visualize] panel to build the image.

First display the [Visualize] panel.

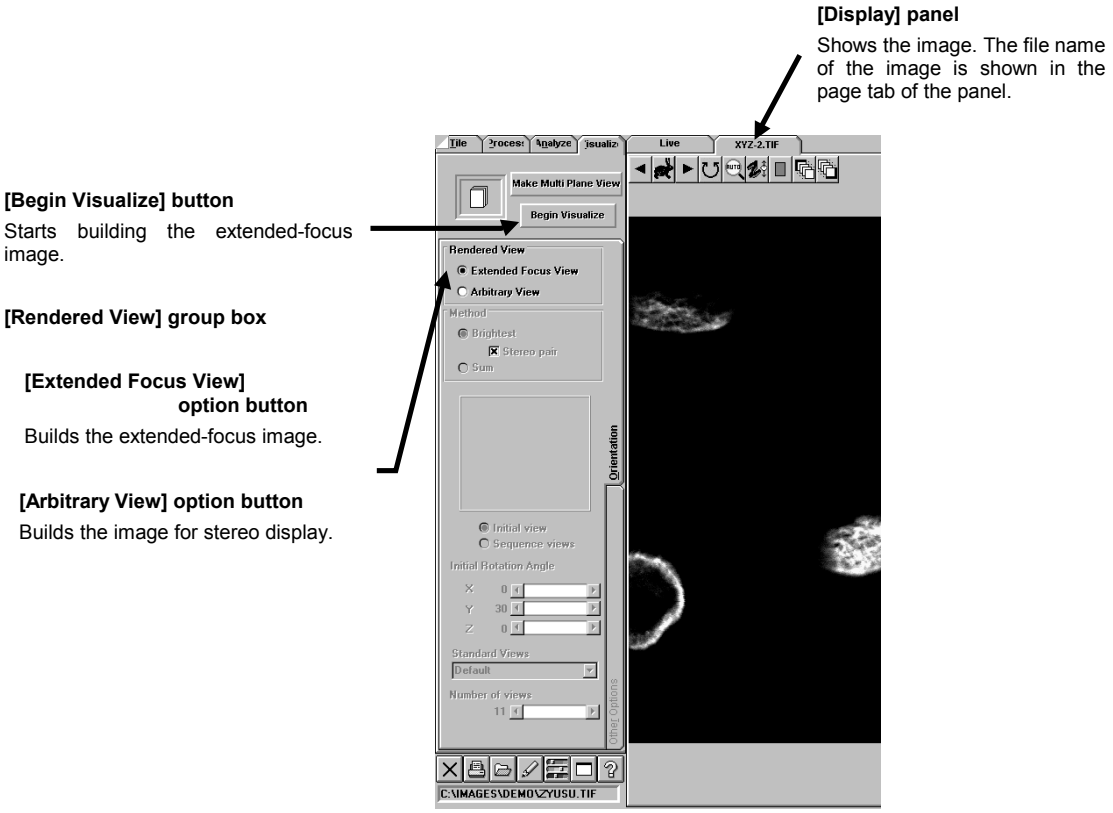


Fig. 2-99 [Visualize] Panel

1. Display the [Display] panel of the XYZ (multiple sections) image.

2. Click the [Extended Focus View] option button in the [Rendered View] group box.

3. Click the <Begin Visualize> button to start the image building. When it completes, the built image is displayed in the [Extended] panel.

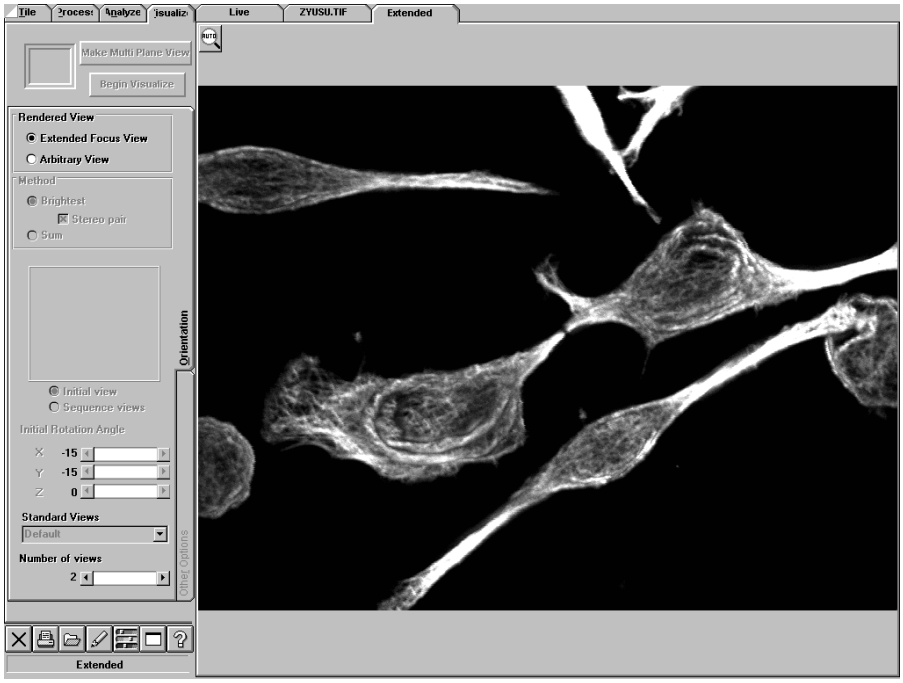


Fig. 2-100 Panel Showing Extended-Focus Image

2-7-1-3 Turning Built Image into time series image

From XYZT image, an extended-focus image can be built as a separate time series image from the original image.

Use the [Visualize] panel to build the image.

First display the [Visualize] panel.

1. Display the [Display] panel of the XYZT image.
2. Click the [Extended Focus View] option button in the [Rendered View] group box.
3. Click the <Begin Visualize> button to start the image building. When it completes, the built image is displayed in the [Extended] panel.

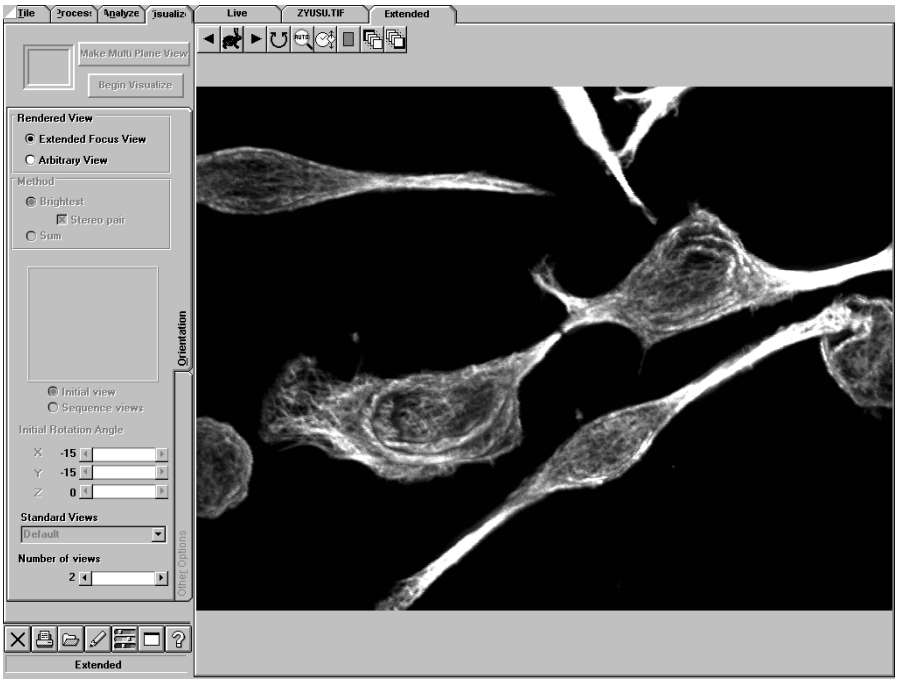


Fig. 2-101 Panel Showing Extended-Focus Image

2-7-2 Building line images to be viewed in Z direction

The images acquired by cutting the XYZ image off vertically and horizontally can be displayed with the information on each line.

Each line images is created as a separate image from the original one.

To display the line images, use the [Visualize] panel.

Display the [Visualize] panel.



<Make Multi Plane View> button

1. Display the [Display] panel of the XYZ (multiple sections) image.
2. Click the <Make Multi Plane View> option button in the [Rendered View] panel.

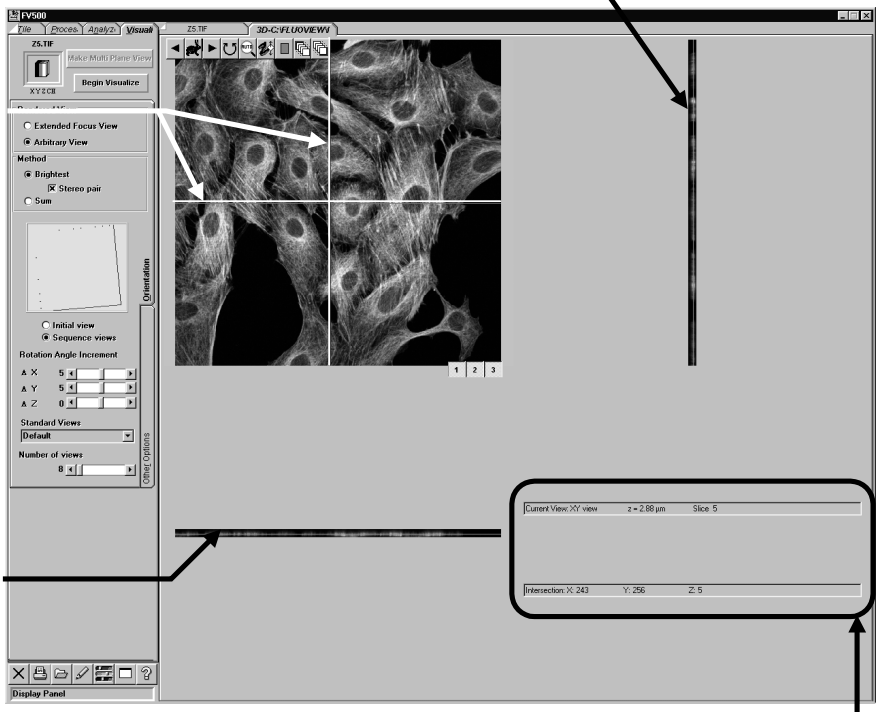
   The [3D-] panel is created to start the line image building.

Line image display in XZ-direction

Display the image of the line specified on the XY-direction image.

Image display in XY-direction

The line image to be displayed in XZ or YZ-direction can be moved by dragging the red line (XZ) or brown line (YZ) on the XY-direction image.



Line image display in YZ-direction

Display the image of the line specified on the XY-direction image.

Fig. 2-102 Panel showing the line images to be viewed in Z direction

Upper row : shows the current slice and the steps in Z-direction acquiring the image.

[z=] : the number of current steps

[Slice] :the number of current slice

Lower row: shows the current X and Y coordinates and Z position on the XY-direction image.

[X:] : current X position

[Y:] : current Y position

[Z:] : current Z position



<Display> button

TIP

If you change the slice to be displayed with the <Display> button, [Slice] and [Z:] aren't effected.

[Slice] and [Z:] show current slice and Z position according to moving the red or brown line indicating XZ or YZ-direction on the XY-direction image displayed left above.

2-8 Viewing 3D Image

Use the [Visualize] panel to view an image three-dimensionally.

First display the [Visualize] panel.

[Begin Visualize] button

Starts building the images for 3D display.

[Method] group box

[Brightest] option button

Builds the image by accumulating the intensity value.

[Stereo Pair] check box

To be checked when building a pair of stereo 3D images or a 3D image to be viewed through color (red/green) eyeglasses.

[Sum] option button

Builds the image by adding the intensity values.

[Initial view] option button

Sets the angle at which the rotation should start. The angle itself can be set using the [Initial Rotation Angle] scale immediately below the option button.

[Sequence views] option button

Sets the rotation angle per rotation step. The angle itself can be set using the [Rotation Angle Increment] scale immediately below the option button.

[Display] panel

Shows the image. The file name of the image is shown in the page tab of the panel.

[Rendered View] group box

[Extended Focus View] option button

Builds the extended-focus image.

[Arbitrary View] option button

Builds the image for 3D display.

[Initial Rotation Angle]/ [Rotation Angle Increment] scales

Set the angles

[Standard Views] drop-down list

Selects the rotation direction. It is possible to set an arbitrary direction.

[Number of views] scale

Sets the number of views displayed during rotation.

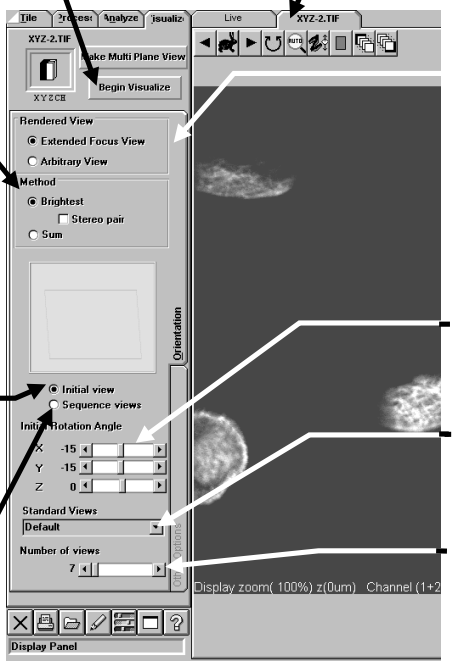


Fig. 2-103 [Visualize] Panel and [orientation] Sub-panel

[Threshold range Low / High] scale

Sets the range of intensity values to be used in image building.

[Depth Weight] scale

Sets the weighting in the depth direction. By increasing this setting, it is possible to provide the image with a perspective by darkening the far objects and brightening near objects.

[Stereo Factor] text box

Sets the deviation between the left and right eyes when building a pair of stereo 3D images or a 3D image to be viewed through color (red/green) eyeglasses.

[Z stretch Factor] text box

Provides each multiple sections image with a feeling of thickness. The value displayed here in advance has been calculated by the system so that the scale in the planar, or XY-direction of the image is identical to the scale in the depth, or Z-direction. Usually, this value does not need to be changed.

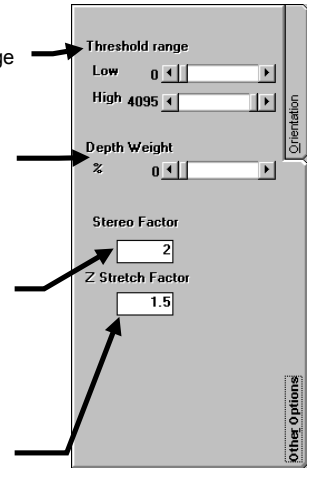
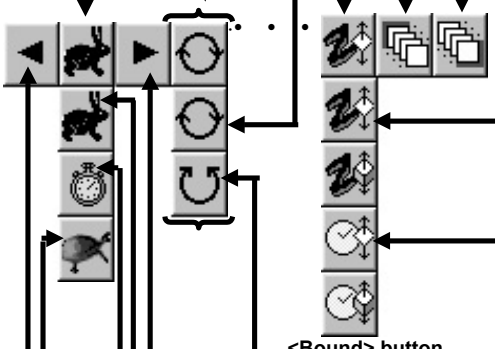


Fig. 2-104 [Other options] Sub-panel of [Visualize] Panel

2-8-1 Successive Display of Images

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation, XYZT observation) can be displayed successively using the buttons at the top of the [Display] panel as shown below.

1. Display the [Display] panel of the image composed of multiple image slices.
2. The buttons as shown below are displayed at the top of the [Display] panel. If the <XYZ series> button is not displayed under the <Z/T series switch> button while the images were acquired in XYZT observation mode, click the <Z/T series switch> button and click the <XYZ series> button in the displayed list of buttons.



<Loop> button

Successive display or frame-by-frame display is repeated in the same direction.

<Loop/Bound switch>button

<Display speed switch> button

Set the speed of successive display.

<Z/T series switch> button

<Set start position> button

When successive display or frame-by-frame display is required, set the image with which the display should start. ("0" is set when this button is not used.)

<Set end position> button

When successive display or frame-by-frame display is required, set the image with which the display should end. (Assuming that the number of images is n, "n-1" is set when this button is not used.)

<XYZ series> button

Displays one of multiple images by selecting it according to the multiple sections (Z).

<XYT series> button

Displays one of multiple images by selecting it according to the time (T).

<Bound> button

Every time successive display or frame-by-frame display in a direction ends at the start or end position, the display is restarted in the opposite direction.

<Display> button

Press to start display of n-slices of image from the start position to the end position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

<Rabbit> button

Press for successive display of image slices without pause.

<Clock> button

Press for successive display of image slices at the time interval set in the [Time Series] sub-panel.

<Turtle> button

Press for successive display of image slices at an interval of 0.8

<Display> button

Press to start display of n-slices of image from the end position to the start position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.

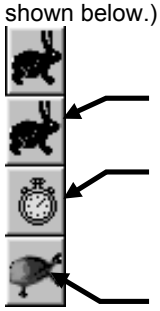
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

3. Click the <Set start position> button. (If the start position is not set, image No. 0 becomes the start image automatically.)
4. Display the image slice to end the successive display by using the <Frame advance> button at the top of the [Display] panel.
5. Click the <Set end position> button. (If the end position is not set, image No. n-1, assuming that the number of images is n, becomes the start image automatically.)
6. Click the <Successive display> button. The images will be displayed successively from the start position to the end position.
   Click the <Stop> button to stop the successive display.

2-8-1-1 Changing the Successive Display Speed

The buttons on the top of the [Display] panel can be used to vary the speed of successive display of multiple image slices.

1. Display the [Display] panel of the image to be subjected to successive display speed change.
2. The buttons as shown below are displayed at the top of the [Display] panel.
   (Usually, the <Rabbit> button is displayed. Clicking it displays a list of buttons as shown below.) Click one of the buttons to select the display speed.

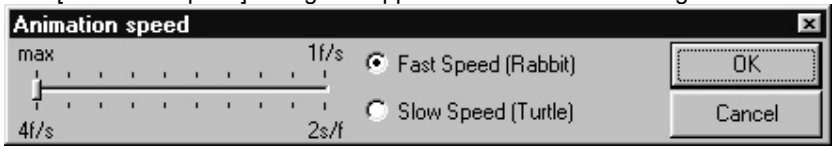


<Rabbit> button
For successive display without pause.

<Clock> button
Press for successive display of image slices at the time interval set in the [Time Series] sub-panel.

<Turtle> button
Press for successive display of image slices at an interval of 0.8 sec.

3. The display speed provided by the <Rabbit> or <Turtle> button can be varied by clicking the mouse right button on each button.
   The [Animation speed] dialog box appears when the button is right-clicked.



4. Select the option button of the speed to be varied.

5. Set the desired display speed in the scale on the right.

6. Click the <OK> button to close the [Animation speed] dialog box.

2-8-2 Animation

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation, XYZT observation) can be built into animation image, which can be displayed in 3D by rotating images.

1. Display the [Display] panel of the image composed of multiple image slices.

2. When the images were acquired in the multi-channel mode, select whether animation is built from images of more than one channel or from an image of only one channel.
   To select the target channel(s), use the <Display channel switch> buttons. Only the channel(s) being displayed will be used in the animation building.
   Example) When only the Ch1 image is displayed, only the Ch1 image is used.



<Display channel switch> buttons

TIP For the switching of channels, see section 2-4-3, “Switching the Display Channels”.

3. Click the [Arbitrary View] option button in the [Rendered View] group box.
4. Click the [Brightest] or [Sum] option button in the [Method] group box.
5. Select the image rotation direction from the [Standard Views] drop-down list.
6. Click the [Initial view] option button.
7. Set the angle at which the rotation should start using the [Initial Rotation Angle] scale.
8. Click the [Sequence views] option button.
9. Set the angle per rotation step using the [Rotation Angle Increment] scale.

10. Set the number of images to be rotating using the [Number of views] scale.
11. Click the <Begin Visualize> button to start building the animation. When the building completes, the built image is displayed in the [3D Animation] panel.

TIP The status bar shows the progress of building processing.



<Cancel Visualize> button

TIP During the building, the <Begin Visualize> button turns into the <Cancel Visualize> button. Click the <Cancel Visualize> button if you want to cancel the building.

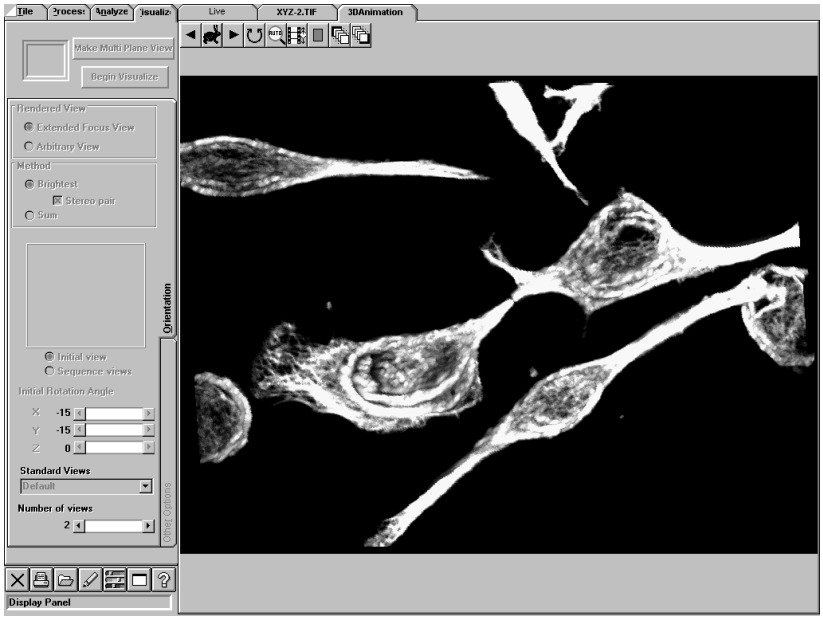


Fig. 2-105 Panel Showing the Built Image


<Display>button

12. Click and hold the <Display> button at the top of the [3D Animation] panel. The images will rotate so that they can be viewed three-dimensionally.

TIP Click the <Stop> button to stop the image rotation.

2-8-3 Building Stereo 3D Images

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation) can be built into a pair of stereo 3D images. These images can be viewed three-dimensionally by watching them with two eyes for a while.

The operation is similar with animation. Perform steps 1 to 10 in section 2-8-2, "Animation", then proceed to the following steps.

1. Check the [Stereo Pair] check box.
2. Click the <Begin Visualize> button to start building the images. When the building completes, the built images are displayed in the [3D Animation] panel.

TIP

The status bar shows the progress of building processing.



<Cancel Visualize> button

TIP

During the building, the <Begin Visualize> button turns into the <Cancel Visualize> button. Click the <Cancel Visualize> button if you want to cancel the building.

3. The following buttons are displayed at the top of the [Display] panel.



Stereo 3D images.

4. Watch the two images with two eyes for a while. They will look as a single 3D image.

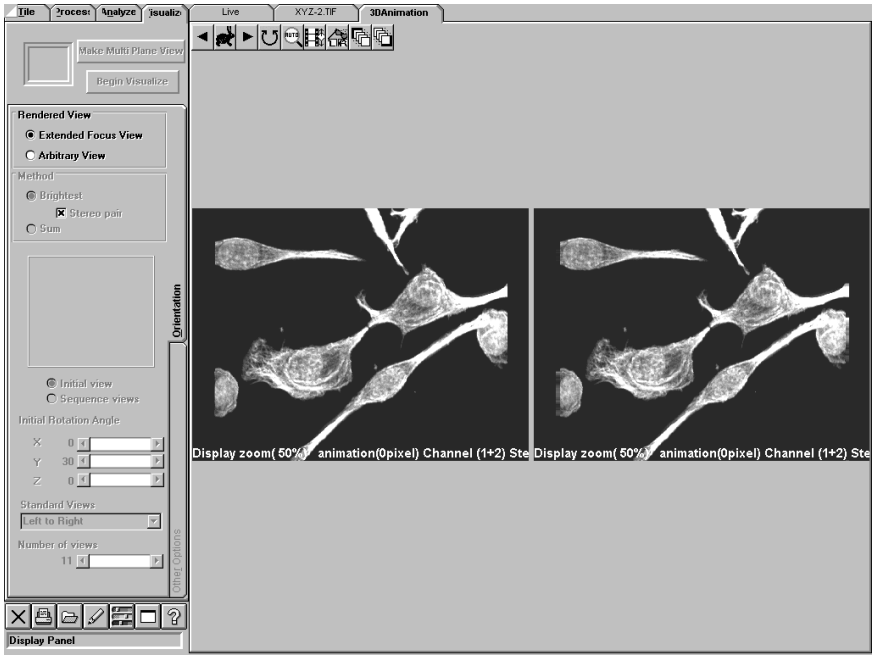


Fig. 2-106 Panel Showing Stereo 3D Images

2-8-4 Building a 3D Image to be Viewed Through Color (Red/Green) Eyeglasses

Images composed of multiple image slices acquired by varying the multiple sections (XYZ observation) can be built into an image which looks 3D when viewed through a pair of color (red/green) eyeglasses. This image can be viewed three-dimensionally by watching them with two eyes for a while.

The operation is similar with animation.. Perform steps 1 to 10 in section 2-8-2, “Animation”, then proceed to the following steps.

1. Check the [Stereo Pair] check box.
2. Click the <Begin Visualize> button to start building the images. When the building completes, the built image is displayed in the [3D Animation] panel.

TIP The status bar shows the progress of building processing.

TIP During the building, the <Begin Visualize> button turns into the <Cancel Visualize> button. Click the <Cancel Visualize> button if you want to cancel the building.

3. The following buttons are displayed at the top of the [Display] panel.



3D image for color (red/green) eyeglasses.

4. Watch the image through a pair of color (red/green) eyeglasses.

TIP The color (red/green) eyeglasses can also be used to view the animation. To start animation, click the <Successive display> button.

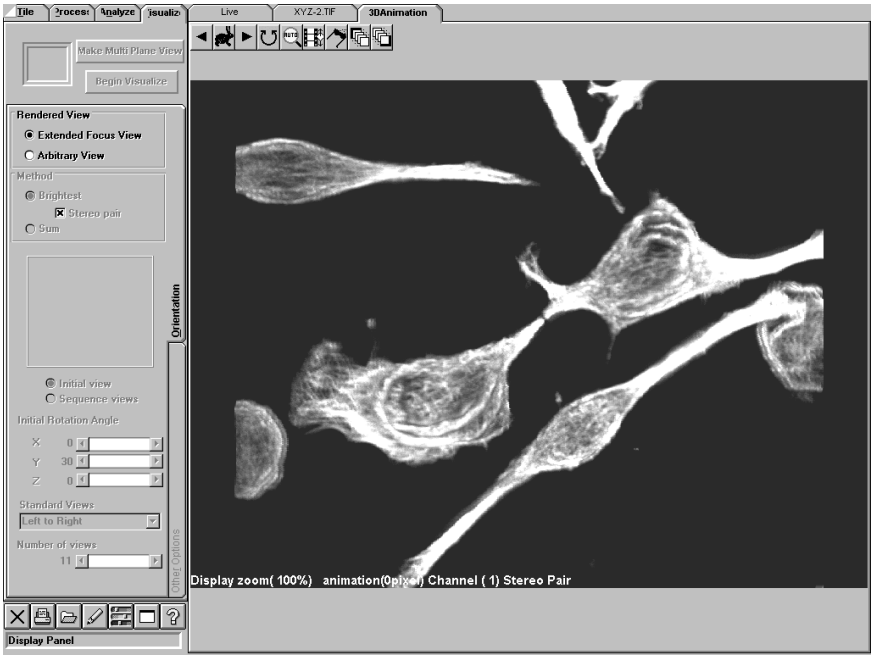


Fig. 2-107 Panel Showing 3D Image to be Viewed Through Color Eyeglasses

2-9 Viewing Images Following the Progress of Time

Images composed of multiple image slices (XYT, XYZT or XZT observation) can be displayed following the time lapse to show the change over time.

2-9-1 Displaying Images Together

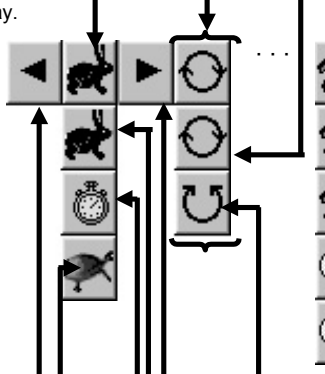
The change over time can be viewed at a glance by displaying multiple image slices together. For the detailed operation method, see section 2-4-7, “Displaying Multiple Image Slices Together”.

2-9-2 Displaying Images Successively

The change over time can also be displayed as animation.

With images composed of multiple slices, such as time-lapse images the buttons as shown below are displayed at the top of the [Display] panel.

1. Display the [Display] panel of the image composed of multiple image slices.
2. To display XYZT observation images by noticing the progress of time, it is required to select the <XYT series> button under the <Z/T series switch> button at the top of the [Display] panel. If the <XYT series> button is not displayed, click the <Z/T series switch> button and click the <XYT series> button in the displayed list of buttons.
3. Display the image slice to start the successive display by using the <Frame advance> button at the top of the [Display] panel.



<Loop> button

Successive display or frame-by-frame display is repeated in the same direction.

<Loop/Bound switch>button

<Display> button

Set the speed of successive display.

<Z/T series switch> button

<Set start position> button

When successive display or frame-by-frame display is required, set the image with which the display should start. (“0” is set when this button is not used.)

<Set end position> button

When successive display or frame-by-frame display is required, set the image with which the display should end. (Assuming that the number of images is n, “n-1” is set when this button is not used.)

<XYZ series> button

Displays one of multiple images by selecting it according to the multiple sections (Z).

<XYT series> button

Displays one of multiple images by selecting it according to the time (T).

<Bound> button

Every time successive display or frame-by-frame display in a direction ends at the start or end position, the display is restarted in the opposite direction.

<Display> button

Press to start display of n-slices of image from the start position to the end position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.
Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame-by-frame.

<Rabbit> button

Press for successive display of image slices without pause.

<Clock> button

Press for successive display of image slices at the time interval set in the [Time Series] button.

<Turtle> button

Press for successive display of image slices at an interval of 0.8 sec.

<Display> button

Press to start display of n-slice of image from the end position to the start position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.

Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

4. Click the <Set start position> button. (If the start position is not set, image No. 0 becomes the start image automatically.)
5. Display the image slice to end the successive display by using the <Display> button at the top of the [Display] panel.
6. Click the <Set end position> button. (If the end position is not set, image No. n-1, assuming that the number of images is n, becomes the start image automatically.)
7. Click the <Display> button. The images will be displayed successively from the start position to the end position.

   To stop the successive display, click the <Stop> button again.

2-10 Transferring Data to Another Application

2-10-1 Transferring Analysis Data to Another Application

Analysis data can be transferred to Excel.

1. Display the [Analyze] panel and executes analysis. After it, display the [Enhanced Profile Plot], [Intensity Map], [Enhanced Histogram Plot], {Average Intensity Trace] or [Integrated Intensity Trace].

TIP

For the operation method, see section 2-6, “Image Analysis”.

<Save> button

Saves the profile data in a file using an Excel-compatible format.

<Close> button

Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

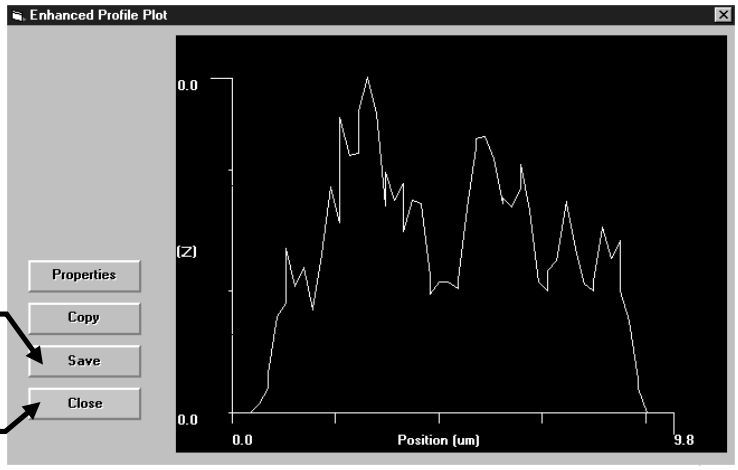


Fig. 2-108 [Enhanced Profile Plot] Window

TIP

Microsoft Excel is not included in the FLUOVIEW FV300 system.

Please purchase it separately.

2. Click the <Save> button. When the [Save As] dialog box appears as shown below, set the file name and click the <OK> button to save the analysis data.

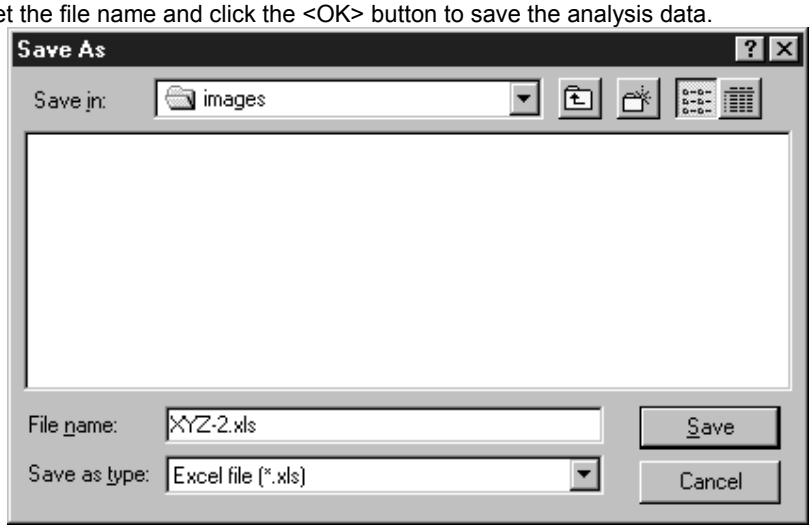


Fig. 2-109 [Save As] Dialog Box

3. Exit from FLUOVIEW or display the [Start] menu by pressing the Ctrl + Esc keys.
4. Select [Programs] and issue the [Microsoft Excel] command.
5. From the [File] menu of Excel, select the [Open] command and open the file saved in step 2.
6. When the dialog box as shown below appears, click the [Delimited] option button in the [Original Data Type] group box, then select [Windows (ANSI)] from the [File Origin:] drop-down list.

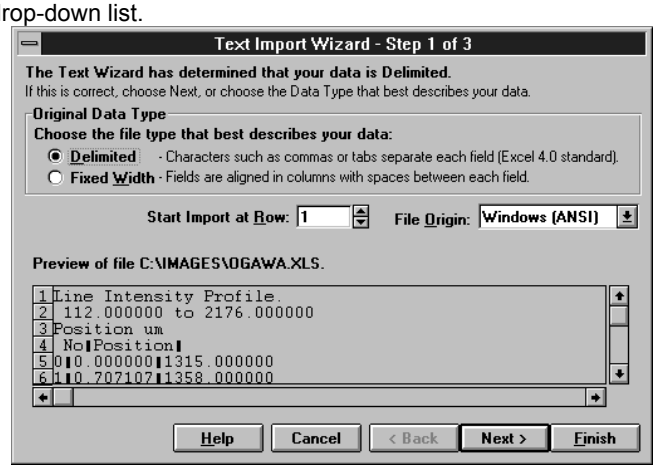


Fig. 2-110 Dialog Box When the File is Opened by Excel 1/3

7. Click the <Next> button. When the dialog as shown below appears, check the [Tab] check box in the [Delimiters] group box, then select [[none]] from the [Text Qualifier:] drop-down list.

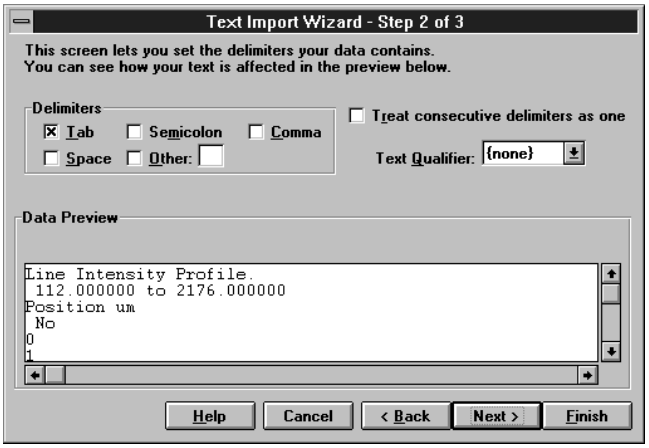


Fig. 2-111 Dialog Box When the File is Opened by Excel 2 / 3

8. Click the <Next> button. When the dialog as shown below appears, select the [General] option button in the [Column Data Format] group box, then click the <Finish> button.



Fig. 2-112 Dialog Box When the File is Opened by Excel 3 / 3

TIP

For detailed operation procedures of Excel, refer to the [Excel manuals].

A1 Line Intensity Profile.

	A	B	C	D	E	F	G	H
1	Line Intensity Profile.							
2	112.000000 to 2176.000000							
3	Position um						0.000000 to 181.048776	
4	No	Position						
5	0	0	1315					
6	1	0.707107	1358					
7	2	1.414214	1353					
8	3	2.12132	1334					
9	4	2.62132	1365					
10	5	3.328427	1404					
11	6	4.035534	1276					

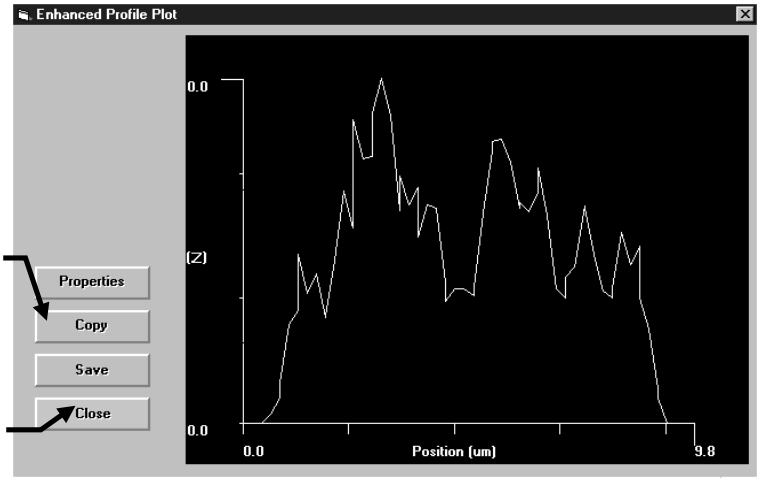
2-10-2 Transferring the Plot Image of Analysis Data to Another Application

The plot image of analysis data can be transferred to an application handling images, such as Paint. The following description takes Paint as example.

1. Display the [Analyze] panel and executes analysis. After it, display the [Enhanced Profile Plot], [Intensity Map], [Enhanced Histogram Plot], {Average Intensity Trace] or [Integrated Intensity Trace].

TIP

For the operation method, see section 2-6, “Image Analysis”.



<Copy> button
Copies the plot image to the clipboard.

<Close> button
Quits the [Enhanced Profile Plot] window and returns to the [Analyze] panel.

Fig. 2-113 [Enhanced Profile Plot] Window

2. Click the <Copy> button to copy the plot image to the clipboard.
3. Exit from FLUOVIEW or display the [Start] menu by pressing the Windows key or the Ctrl + Esc keys.
4. Select [Programs]-[Accessories] and issue the [Paint] command.
5. From the [Edit] menu of Paint, select the [Paste] command and paste the plot image which has been copied to the clipboard in step 3.

TIP

For detailed operation procedures of Paint, refer to the [help provided by Paint].

2-10-3 Transferring Image Data to Another Application (microVoxel, Paint, etc.)

To transfer image data to another application, the image data should be saved in a file and the file should be transferred.

1. Save an image using one of the formats that can be handled by the destination application. See sections 2-3, “Saving, Opening and Shredding Images” and 2-3-1-1, “Saving Images As a Series” for the image saving procedure.

2. Exit from FLUOVIEW or display the [Start] menu by pressing the Windows key or the Ctrl + Esc keys.

NOTE

To transfer data to microVoxel, it is required to exit from FLUOVIEW, exit from Windows and restart OS/2.

3. Start the image transfer destination application (Paint, etc.).

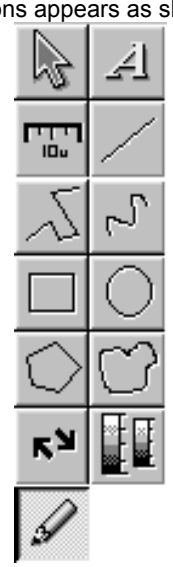
4. In the application, open the file saved in step 1.

2-11 Entering Comment in Image

Comment can be entered in an image for use in presentation or slide creation.


<Annotate> button

Use the <Annotate> button in the toolbar at the bottom left of the screen. Click the <Annotate> button. A list of buttons appears as shown below.



2-11-1 Writing Characters in Image

This facility is used to enter the title, acquisition parameters and/or notes in an image. Some labels are provided in advance. Characters can be written either by using these labels or entering desired characters at will.


<Text> button

1. Display the [Display] panel of the image in which you want to write characters.
2. Click the <Text> button in the displayed list of buttons. The dialog as shown below appears.

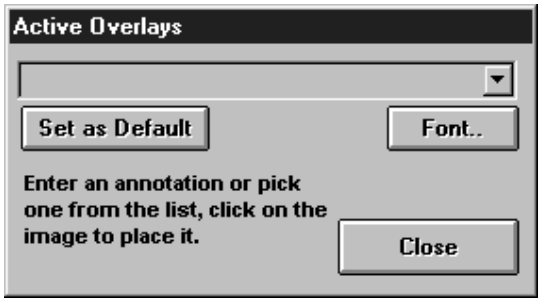


Fig. 2-114 [Active Overlays] Dialog Box

3. From the drop-down list in the dialog box, select one of the following labels.
   - Z Position <z>
   - Time Instant <t>
   - Channel # <channel>
   - Display Zoom <display zoom>
   - Is bounded <bounds>
   - Experiment Name <name>
   - <display zoom> <z> <t> <animation> <channel> <stereo> <bounds>

TIP

Desired characters can also be entered.

1. Click the character in the drop-down list of the dialog box.
2. Delete the character by pressing the Delete or Back Space key
3. Enter characters from the keyboard.

TIP

When the <Set as Default> button is pressed after having selected a label, it is set as the label displayed permanently at the bottom left of the [Display] panel.

4. Click the <Font> button. The dialog box as shown below appears.

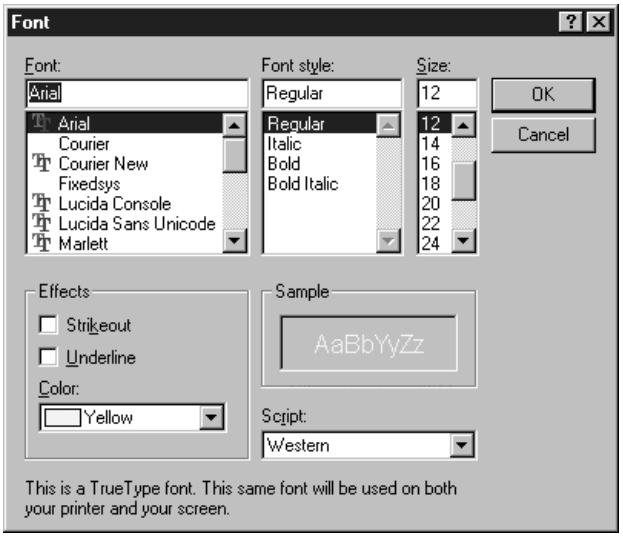


Fig. 2-115 [Font] Dialog Box

5. Select the character font and size using the [Font], [Font Style] and [Size] list boxes.
6. Click the <OK> button to close the [Font] dialog box.
7. Click the <Close> button to close the [Active Overlays] dialog box.
8. Place and click the mouse pointer on the image position you want to enter characters.

TIP

Refer to “Appendix J List of Functions in the [Active Overlays] Dialog Box” for other functions of the [Active Overlays] dialog box.

2-11-2 Displaying the Image Intensity

The intensity of any pixel of an image can be displayed without using the [Analyze] panel.

1. Display the [Display] panel of the image that you want to display the intensity at the front.


<Text> button

2. In the list of buttons displayed, click the <Text> button. The dialog box as shown below appears.

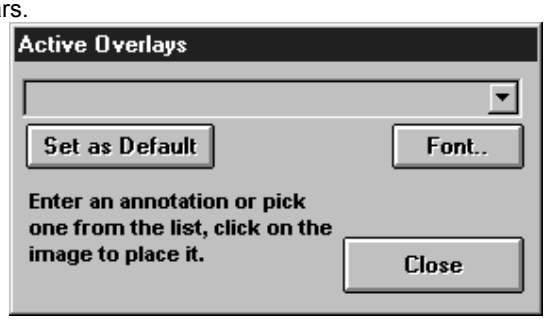


Fig 2-116 [Active Overlays] Dialog Box

3. In the drop-down list inside the dialog box, select” I = <intensity hotspot value>“.
4. On the image, place the mouse pointer on the position that you want to display its intensity and click the mouse.
5. Click the <Close> button to close the [Active Overlays] dialog box.

2-11-3 Displaying the X-coordinate/Y-coordinate of the Image

The X-coordinate position or the Y-coordinate position of any pixel of an image can be displayed.


<Text> button

1. Display the [Display] panel of the image that you want to display the X-coordinate position or the Y-coordinate position at the front.

2. In the list of buttons displayed, click the <Text> button. The dialog box as shown below appears.

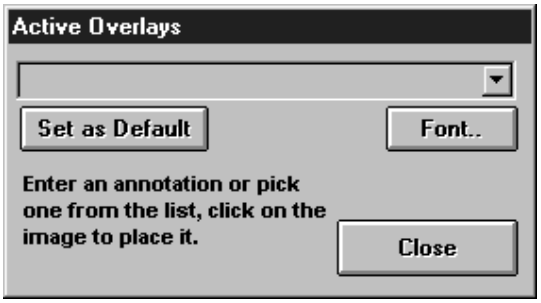


Fig 2-117 [Active Overlays] Dialog Box

3. In the drop-down list inside the dialog box, select “x = <x hotspot value “” units> “ to display the X-coordinate position or “Y = hotspot value “” units>“ to display the Y-coordinate position.

4. On the image, place the mouse pointer on the position that you want to display the X-coordinate position or the Y-coordinate position and click the mouse.

5. Click the <Close> button to close the [Active Overlays] dialog box.

TIP

The displayed value indicates the distance from the origin when assuming the upper left of the image as the origin.

2-11-4 Drawing a Figure in Image

This facility is used to draw figures in the image. FLUOVIEW provides seven figure drawing modes.

1. Display the [Display] panel of the image in which you want to draw figures.
2. Select one of the following command buttons and draw a figure in the image using the mouse. The operation methods of the command buttons are described below.


<Line> button

To draw a straight line:
On the image, click the point that you want to start the straight line, and drag the mouse from there to the point you want to end it.


<Poly Line> button

To draw a polygonal line:
On the image, click the points corresponding to the start point, peak points and end point of the desired polygonal line, then click the right button of the mouse to set the specification.


<Rectangular> button

To draw a rectangle:
On the image, drag the mouse pointer along the diagonal line of the desired rectangle, from the top left corner to the bottom right corner.


<Circle> button

To specify a circle or ellipse:
On the image, assume a rectangle circumscribing the circle to be checked and drag the mouse pointer along the diagonal line between opposite corners of the rectangle.


<Polyregion> button

To specify a polygonal region:
On the image, click the points corresponding to the corners of the desired polygon. After clicking the last corner point, click the right button of the mouse to connect the last clicked point to the first clicked point.


<Free Region> button

To specify a free region:
On the image, specify a region by dragging. Then release the mouse button to complete dragging. The point where the dragging was ended will be connected to the point where it was started.


<Free Line> button

To specify a free line:
On the image, drag the mouse pointer along the desired line.

2-11-5 Drawing a Scale in Image



<Scale> button

A scale can be drawn between two points in an image.

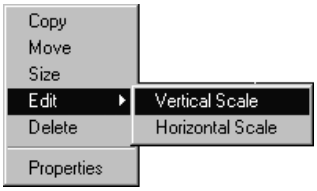
1. Display the [Display] panel of the image in which you want to draw a scale.
2. Click the <Scale> button in the displayed list of buttons.
3. Click the image position you want to draw a scale.
4. Change the scale size. See 2-11-8, “Changing the Comment Size” for the operation procedure.

NOTE **To display a correct scale, it is required that the image has been acquired while the objective magnification setting in the software matches the magnification of the actually used objective.**

TIP

A vertical scale can be drawn as well as a horizontal scale.

1. Click the mouse on the scale to turn the scale active (i.e. handles displayed around the scale).
2. Click the right mouse button.
3. Select [Edit] from the displayed menu. A sub-menu as shown below appears.



4. Select [Vertical Scale] to display a vertical scale.


<Text> button

TIP

The size of characters in the scale can also be changed.

The size of characters in the scale is determined by the size of characters written using the <Text> button. Perform the following operation before starting to draw the scale.

Click the <Text> button in the list of buttons.

1. When the <Annotation> dialog box is displayed, click its <Font> button. The [Font] dialog box will appear.
2. Change the character font and size using the [Font], [Font Style] and [Size] list boxes.
3. Click the <OK> button to close the [Font] dialog box.
4. Click the <OK> button to close the [Annotation] dialog box.

2-11-6 Drawing an Arrow in Image

This facility is used to draw an arrow for indicating a point in interest in image or adding explanation in it.

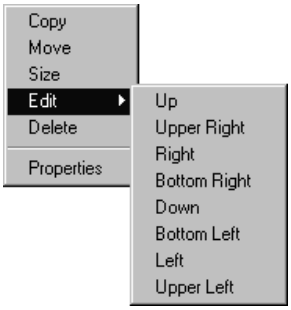

<Arrow> button

1. Display the [Display] panel of the image you want to draw an arrow.
2. Click the <Arrow> button in the displayed list of buttons.
3. Drag the mouse pointer from the start point to the end point of the desired arrow.
4. To change the arrow size, see section 2-11-8, “Changing the Comment Size”.

TIP

The direction indicated by a previously drawn arrow can be changed.

1. Click the mouse on the arrow to make the arrow active (i.e. handles displayed on the arrow).
2. Click the right button of the mouse.
3. Select [Edit] from the displayed menu. A sub-menu as shown below appears.



4. Select the desired arrow direction from the sub-menu.

2-11-7 Drawing Color Bars in Image


<Color Bar> button

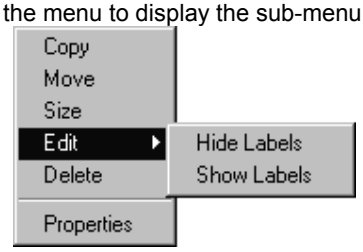
This facility is used to draw color bars in an image.

1. Display the [Display] panel of the image you want to draw color bars.
2. Click the <Color Bar> button in the displayed list of buttons.
3. Draw color bars in the image by dragging the mouse pointer along the desired position in the image.
4. To change the color bar size, see section 2-11-8, “Changing the Comment Size”.

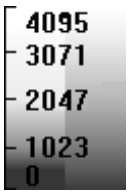
TIP

The labels of the color bars can be switched to display or hide.

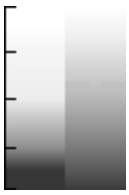
1. Click the mouse on/inside the color bars to select. (The handles appear arround the color bars.)
2. Right-click the mouse.
3. Select [Edit] in the menu to display the sub-menu as shown below.



4. In the sub-menu, select “Show Labels” to display the labels or “Hide Labels” to hide the labels.

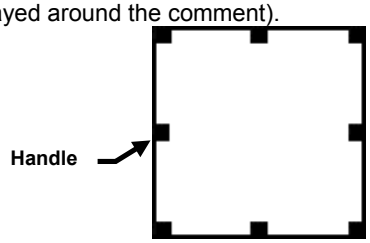


The labels are shown


The labels are hidden

2-11-8 Deleting Comment

1. Click the mouse on the comment to be deleted to make the comment active (i.e. handles displayed around the comment).



2. Click the right button of the mouse. A pop-up menu as shown below appears.

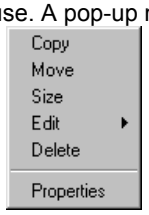


Fig. 2-118 Pop-up Menu

3. Select [Delete] from the menu.

TIP

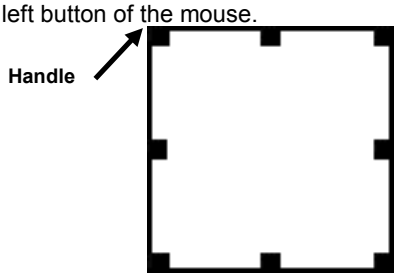
To delete more than one comment simultaneously:
Select multiple comments and select [Delete] from the pop-up menu.
To select, click the mouse on or in the middle of one of the comments and click the moue while holding the Shift key to select the second comment and after.
After making all of the comments to be deleted active (i.e. handled displayed around them), click the right button of the mouse on one of the comments and select [Delete] from the displayed pop-up menu.

2-11-9 Moving Comment

1. Click the moues on the comment to be moved to make it active (i.e. handles displayed around it).
2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-118 appears.
3. Select [Move] from the menu.
4. The mouse pointer turns into a cross (+). Move the mouse to move the comment together with the mouse pointer.
5. Click the left button of the mouse to determine the new position.

TIP

A comment can also be moved by selecting it, placing the mouse pointer on it so that the mouse pointer turns into a cross (+), then dragging the mouse. In this case, the final positioning of the comment can be determined by placing the mouse pointer outside the areas enclosed by the handles and clicking the left button of the mouse.

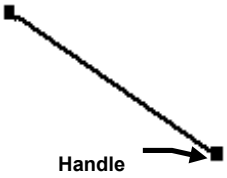


NOTE

The mouse pointer may be hardly visible depending on the image being displayed. In this case, use the method of displaying the pop-up menu.

2-11-10 Changing the Comment Size

1. Click the mouse on the comment to be resized to make it active (i.e. handles displayed around it).
2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-118 appears.
3. Select [Size] from the menu.
4. The menu pointer turns into ↕ When the mouse is moved, the comment is magnified or reduced according to the mouse pointer.
5. Click the left button of the mouse to determine the size.



TIP

A comment can also be magnified or reduced by selecting it, placing the mouse pointer on one of the handles so that the mouse pointer turns into ↕, then dragging the mouse.

2-11-11 Changing the Comment Color

1. Click the mouse on the comment to be changed of color to make the comment active (i.e. handles displayed around it).
2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-118 appears.
3. Select [Properties] from the menu. The [Properties] dialog box as shown below appears. Display the [Color] panel at the front.

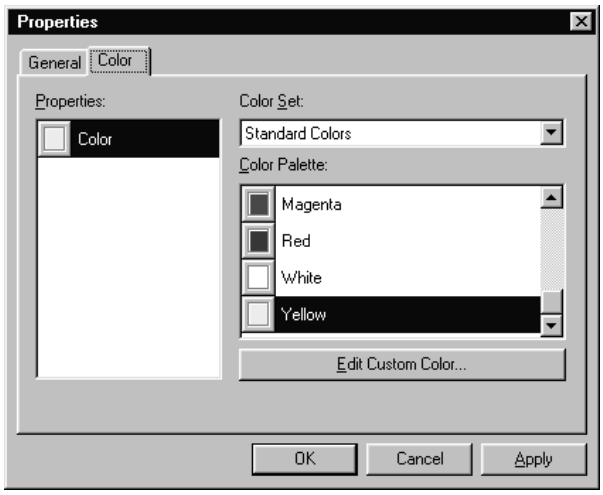


Fig. 2-119 [Properties] Dialog Box

4. Select the desired color from the [Color Palette] list box.

TIP

To change the comment color automatically, set in the [FLUOVIEW Setup] dialog box.
For details, see the description on the [Software] panel in section 1-3-1 in MAINTENANCE, “Overall Setting of FLUOVIEW” for detailed operations.

2-11-12 Changing the Comment Font

1. Click the mouse on the comment to be changed of font to make the comment active (i.e. handles displayed around it).
2. Click the right button of the mouse. A pop-up menu as shown in Fig. 2-118 appears.
3. Select [Properties] from the menu. The [Properties] dialog box as shown below appears. Display the [Font] panel at the front.

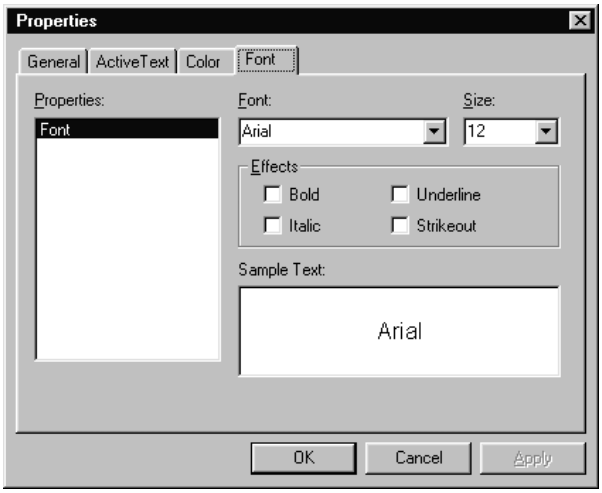


Fig 2-120 [Properties] Dialog Box

4. Set the font type and size using the drop-down lists and select the effect.

2-12 Image Output at Printer


<Print> button

1. Display the [Display] panel of the image to be output at the printer.
2. Click the <Print> button in the toolbar at the bottom left of the panel. A dialog box as shown below appears.

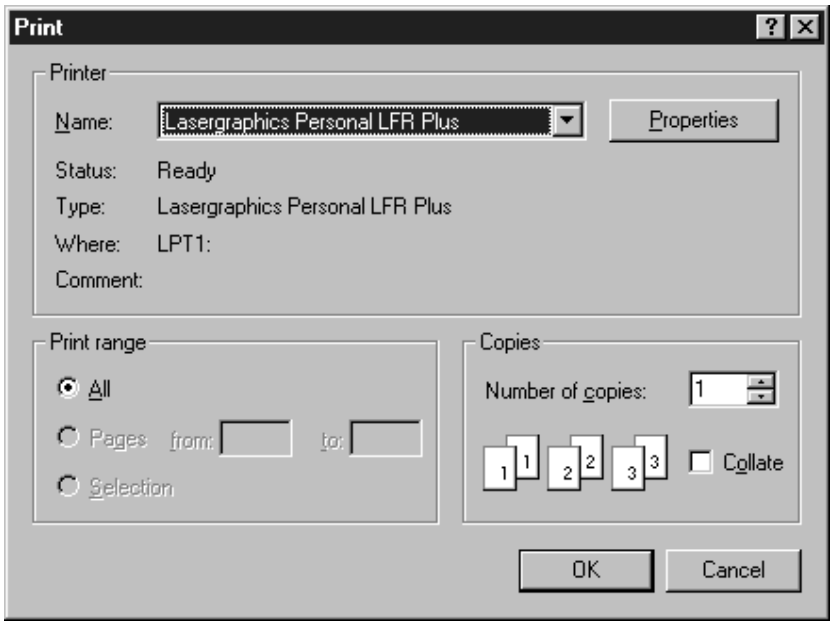


Fig. 2-121 [Print] Dialog Box

3. Select the connected printer name from the [Name] drop-down list.
4. If it is required to set the detailed data of the printer, click the <Properties> button in the dialog box.
5. Click the <OK> button of the dialog box.

NOTE

It is required to install and select the printer driver before the above operation. Refer to the Windows manuals for details.
For the printer operation procedures, refer to the printer manuals.

One Point!

The [Print] dialog box can also be displayed by mouse operation on the image.

1. Display the image to be output at the printer at the front of the [Display] panel, and click the right button of the mouse on the image.
2. A pop-up menu as shown below appears.
3. Select [Print] from the menu.

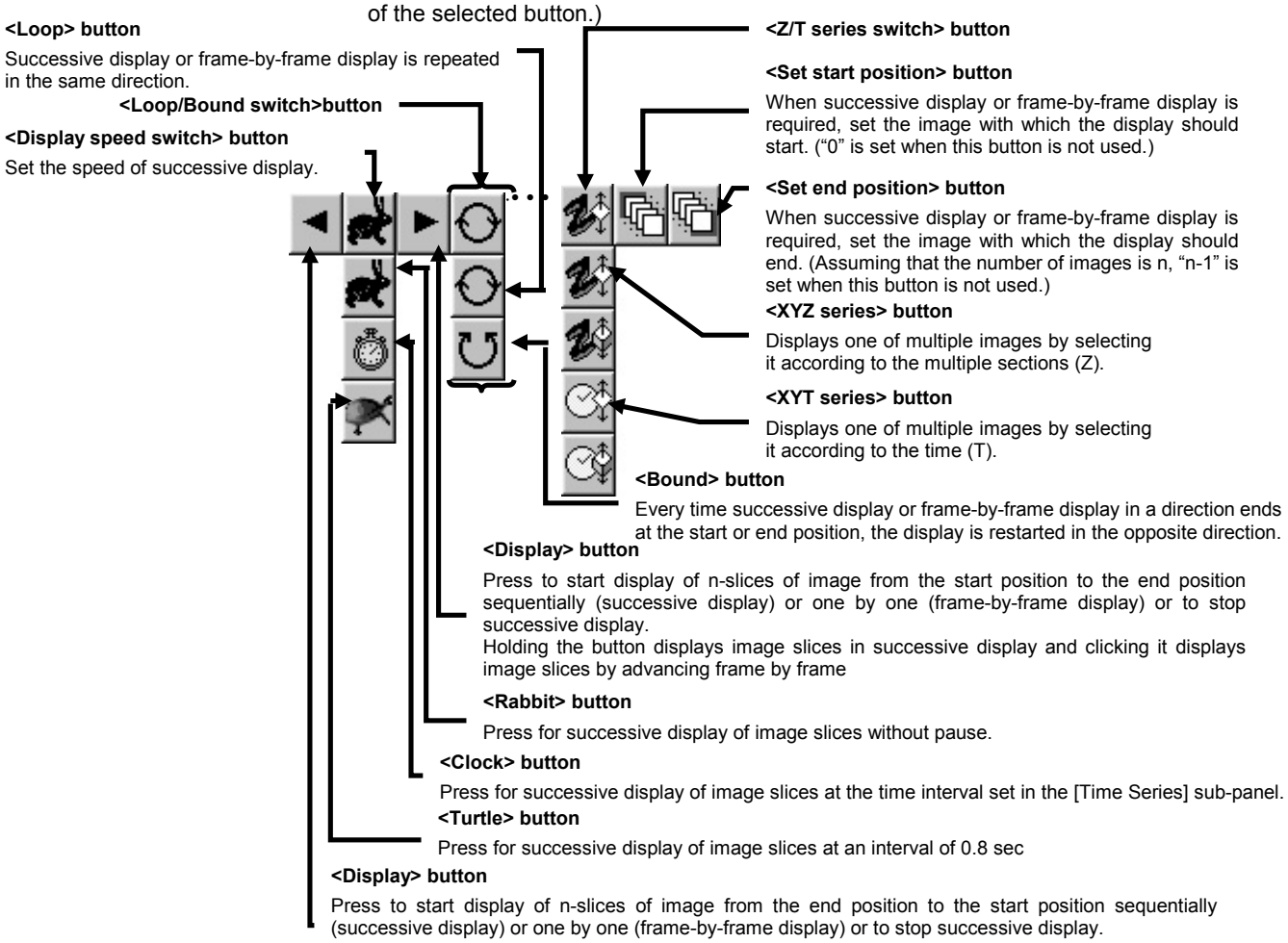


2-13 Merger/Extraction of Image Channels

2-13-1 Setting the Range of Multiple Image Slices

When merging or extracting channels in images composed of multiple image slices, such as time-lapse images and images acquired by varying the cross-sections, it is possible to select only some of image slices as the target of channel merger or extraction. This section describes how to set the range of target image slices.

1. Display the [Display] panel of the images composed of multiple image slices.
2. The buttons as shown below are displayed at the top of the [Display] panel. To switch to the image slice of another cross-section, click the <Z/T series switch> button then click the <XYZ series> button in the displayed list of buttons. To switch to the image slice of another instant in the elapsed time, click the <Z/T series switch> button then click the <XYT series> button in the displayed list of buttons. (In these operations, the icon in the <Z/T series switch> button changes to the icon of the selected button.)



3. Display the image slice to start the range at the front using the <Display> button at the top of the [Display] panel.
4. Click the <Set start position> button. (If the start position is not set, image No. 0 becomes the start image automatically.)
5. Display the image slice to end the range at the front using the <Display> button.
6. Click the <Display> button. (If the end position is not set, image No. n-1, assuming the number of image slices is n, becomes the start image automatically.)

2-13-2 Merging Image Channels

For instance, when a specimen is imaged with two scans for 2-channel fluorescence observation and 1-channel transmitted light observation, the transmitted light image can be merged to the fluorescence images to create a 3-channel image. Note that the display gradation of the images obtained by overlaying 3 channels may be poor than original.

1. Open the two files to be used in creating a new image.

NOTE

The image files used in overlay are subjected to the following restrictions.

· The sizes of the images in the two image files should be identical.

· The number of data bits in the two image files should be identical.

(For example, it is not possible to overlay a Fluoview Multi Tiff file with a Single TIF(S) 8-bit file.)

TIP

When the image in one of the image files is composed of multiple image slices, it is possible to use only some of the slices by setting a slice range. See section 2-13-1, “Setting the Range of Multiple Image Slices” for the operation procedure.

When the images in both image files are composed of multiple image slices, the overlaid image will have the same number of image slices as the file with the fewer image slices.

Example 1: XYZ image + XY image = XY image

(The first image slice of the XYZ image is overlaid with the XY image.)

Example 2: XYZ image with 10 slices + XYZ image with 5 slices = XYZ image with 5 slices

(The first five image slices are overlaid.)


<Experiment List> button

2. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.



Fig. 2-122 [Experiments in Memory] Dialog Box



<ADV> button

3. Click the <ADV> button in the [Experiments in Memory] dialog box. The [Experiment Editor] group box appears as shown below.

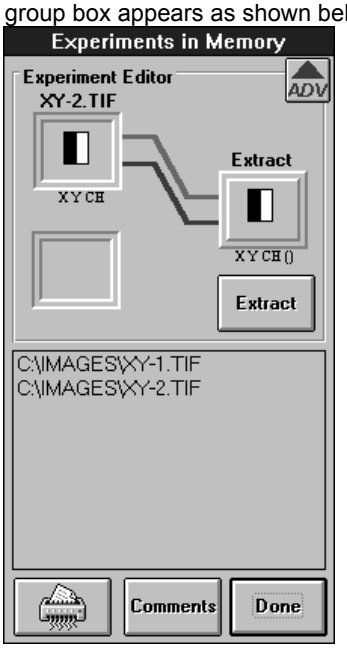
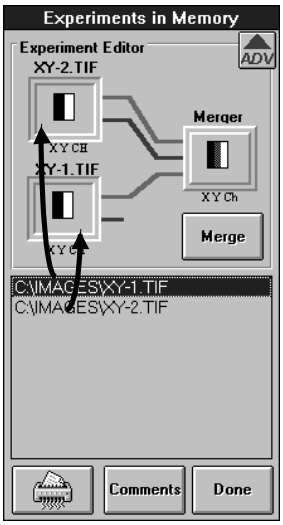


Fig. 2-123 [Experiment Editor] Group Box Displayed by <ADV> Button

TIP

The frame at the top left of the [Experiment Editor] group box shows the icon of the image file displayed at the front of the [Display] panel.



4. From the file list in the [Experiments in Memory] dialog box, select the file name of the first image and drag it into the frame at the top left of the [Experiment Editor] group box. The icon of the first image is displayed in the frame at the top left of the [Experiment Editor] group box.

TIP The mouse pointer turns into the image icon during dragging.

5. From the file list in the [Experiments in Memory] dialog box, select the file name of the second image and drag it into the frame at the top left of the [Experiment Editor] group box. The icon of the second image is displayed in the frame at the top left of the [Experiment Editor] group box.

TIP The mouse pointer turns into the image icon during dragging.

NOTE **When the image of the second selected image file is composed of multiple image slices, the setting of the image slice range is ineffective even when the range is set.**

6. Up to 3 channels are selected automatically beginning with the first image set in the [Experiment Editor] group box, and connected to [Merger] by lines.

NOTE

The lines of channels are colored as shown below.

Ch1: Navy blue

Ch2: Light blue

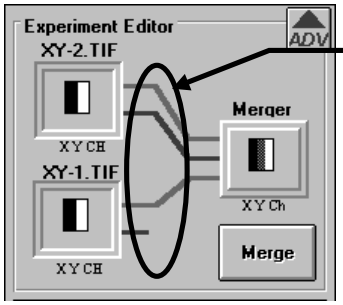
Ch3: Green

Ch4: Yellow green

Ch5: Orange

Ch6: Red

TIP When the mouse pointer is approached to the icon-side end of a line connected to [Merger], the color of the line end turns into yellow. Clicking the line in this condition switches the channel between the selected and deselected status alternately. The lines connected to [Merger] indicate the selected channels.



Clicking a channel line in this area switches the channel between the selected and deselected status.

NOTE

Up to 6 channels can be selected together.

To select another channel after having selected 6 channels, deselect the unnecessary channels before selecting required channels.

7. Click the <Merge> button in the [Experiment Editor] group box. A new [Display] panel showing [Merge] in the page tab appears and the images including newly merged channel(s) are displayed in the panel.

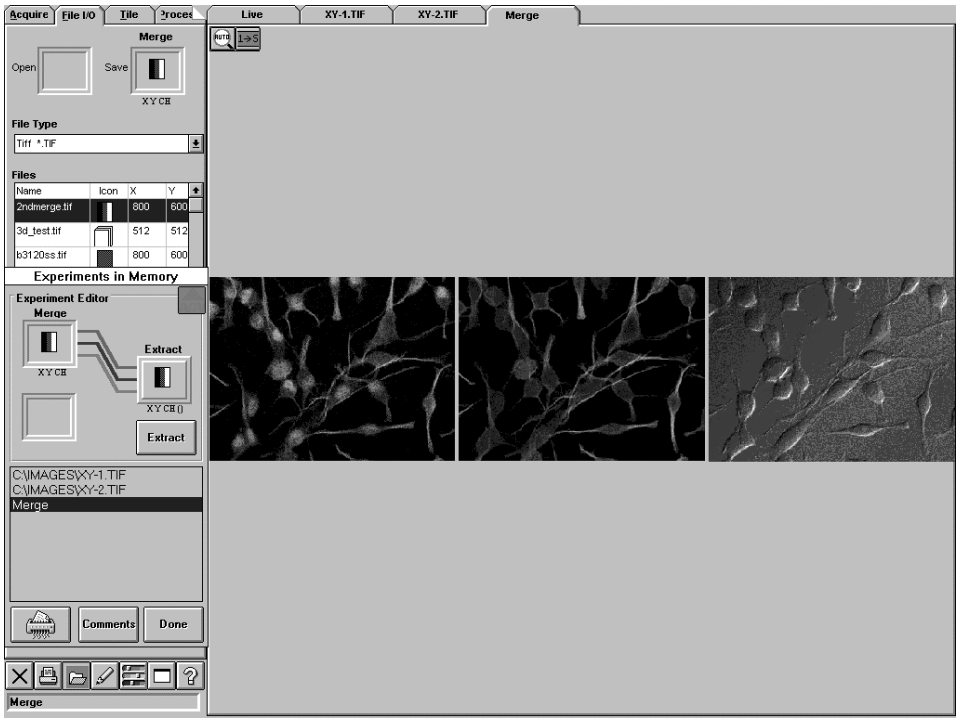


Fig. 2-124 [Merge] Panel

2-13-3 Extracting Channels from Image

Desired channels can be extracted from an image. Use this facility to extract only the images of required channels from an image acquired from more than one channel.

1. Open the image file of the image to extract channels in advance.

TIP

When the image has been saved in more than one image file, it is possible to use only some of the slices by setting a slice range. See section 2-13-1, "Setting the Range of Multiple Image Slices" for the operation procedure.

NOTE

The channel extraction cannot change the image type (XYZ, XYT, etc.). Therefore, it is not possible, for example, to extract an XY image from an XYZ or XYT image.



<Experiment List> button

2. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.



Fig. 2-125 [Experiments in Memory] Dialog Box



<ADV> button

3. Click the <ADV> button in the [Experiments in Memory] dialog box. The [Experiment Editor] group box appears as shown below.

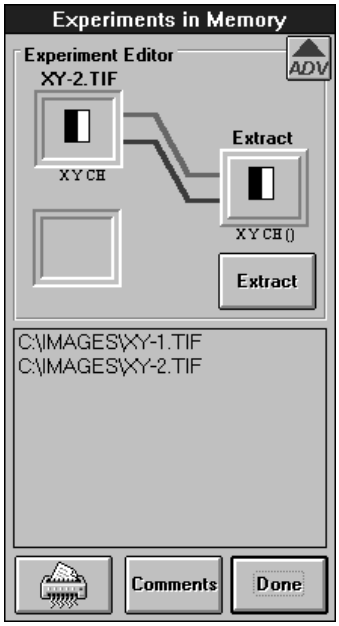
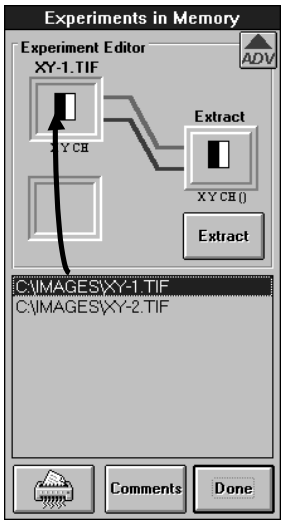


Fig. 2-126 [Experiment Editor] Group Box Displayed by <ADV> Button

TIP The frame at the top left of the [Experiment Editor] group box shows the icon of the image file displayed at the front of the [Display] panel.



4. From the file list in the [Experiments in Memory] dialog box, select the file name of the image from which to extract channels and drag it into the frame at the top left of the [Experiment Editor] group box. The icon of the image is displayed in the frame at the top left of the [Experiment Editor] group box.

TIP The mouse pointer turns into the image icon during dragging.

5. The channels of the image set in the [Experiment Editor] group box are connected to [Extract] by lines.

NOTE

The lines of channels are colored as shown below.

Ch1: Navy blue

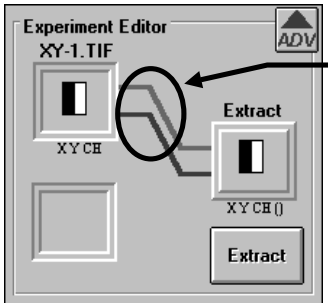
Ch2: Light blueCh3: Green

Ch4: Yellow green

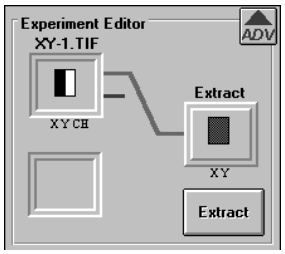
Ch5: Orange

Ch6: Red

TIP When the mouse pointer is approached to the icon-side end of a line connected to [Extract], the color of the line end turns into yellow. Clicking the line in this condition switches the channel between the selected and deselected status alternately. The lines connected to [Extract] indicate the selected channels.



Clicking a channel line in this area switches the channel between the selected and deselected status.



6. Among the channel lines connected to [Extract], deselect the unnecessary channels as described in the TIP on the previous page so that only the necessary channels are selected.

7. Click the <Extract> button in the [Experiment Editor] group box. A new [Display] panel showing [Extract] in the page tab appears and the image of the extracted channel(s) is displayed in the panel.

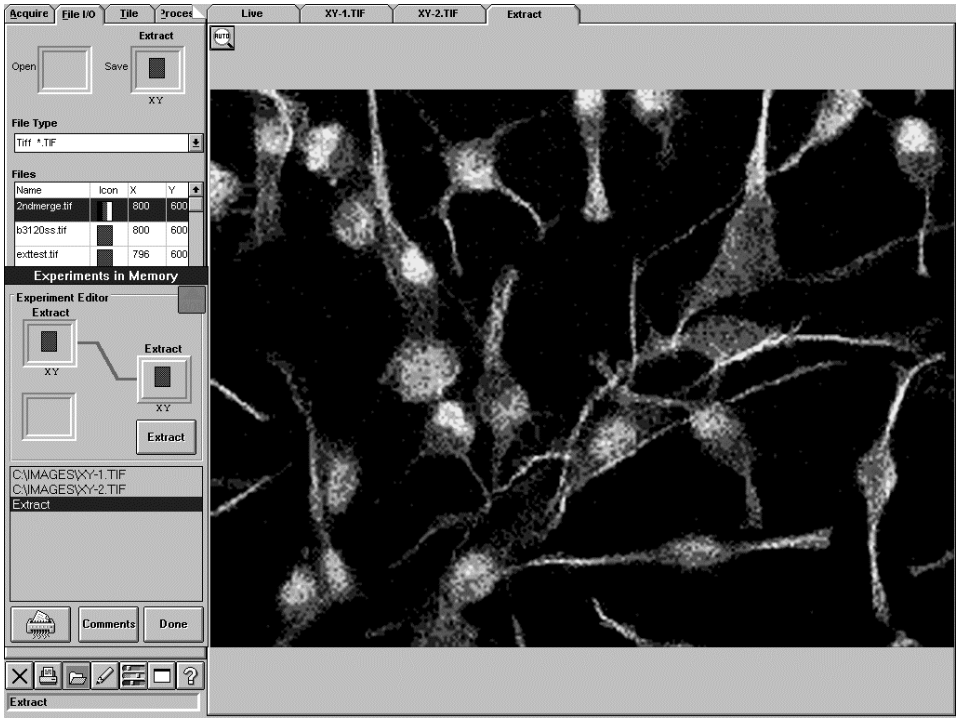


Fig. 2-127 [Extract] Panel

2-14 Changing the Chart Display Method

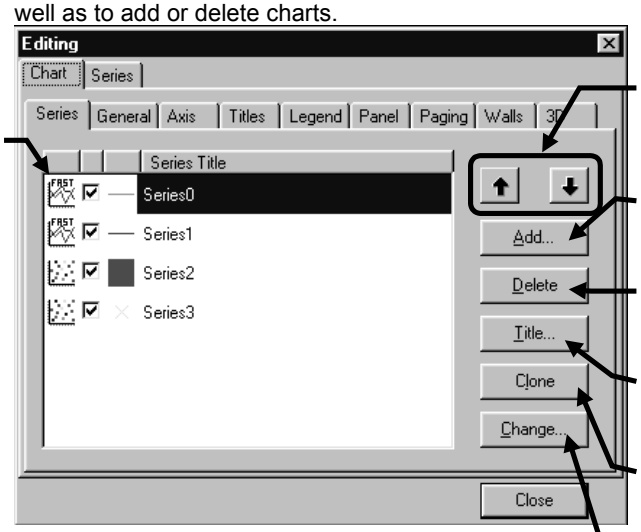
When the processed analysis data chart in the [Analyze] panel is double-clicked, the [Enhanced Profile Plot] dialog box appears.

By clicking the <Properties> button, the [Editing] dialog box can be displayed, allowing the detailed chart settings and chart display method to be changed.

2-14-1 [Chart] Panel

- [Series] panel

  This panel is used to change the type, title or other settings of the displayed chart as well as to add or delete charts.

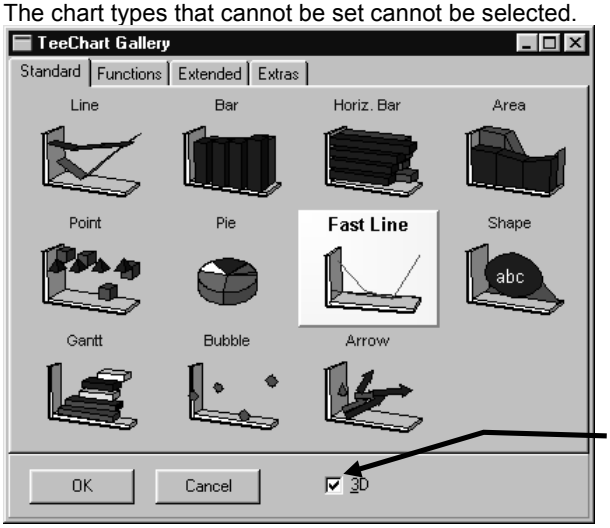


- [TeeChart Gallery] sub-panel

  Displayed when the <Add> or <Change> button is clicked.

  Used to set the chart type.

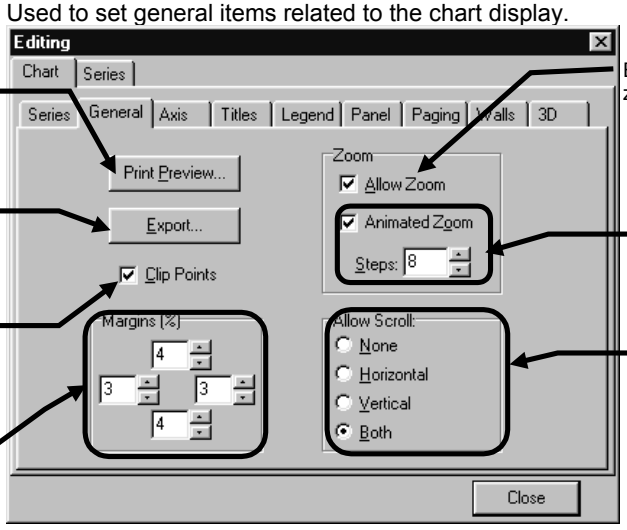
  The chart types that cannot be set cannot be selected.



Enables or disables the 3D display.

- [General] sub-panel

  Used to set general items related to the chart display.



Previews the printed chart or sets the orientation and margins of the print paper.

Saves the chart in the format of an image file or in the clipboard.

When a chart is displayed by zooming or scrolling, selects whether the data of only the area contained in the chart axes is to be displayed or not.

Sets the margins from the edges of the area in which the chart is displayed.

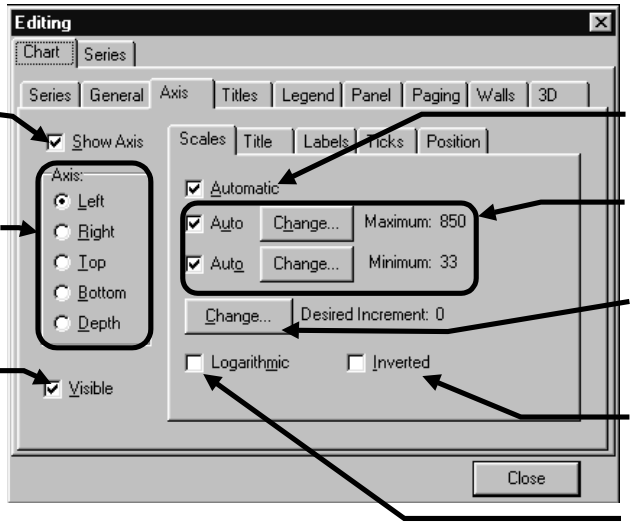
Enables or disables zooming.

When zooming is enabled, enables or disables gradual zooming according to the step count set in the [Steps:] dialog box.

Sets the chart scrolling direction.

- [Axis] sub-panel

Used to set the coordinate axes of the chart.



Enables or disables the display of chart axes.

Selects the chart axes to be set in the [Scales], [Title], [Labels], [Ticks] and [Position] panels.

Enables or disables the chart axis display set in the [Axis:] group box.

Displays the chart axes by selecting the optimum scale automatically.

Sets the maximum and minimum values of the chart axes either automatically or manually.

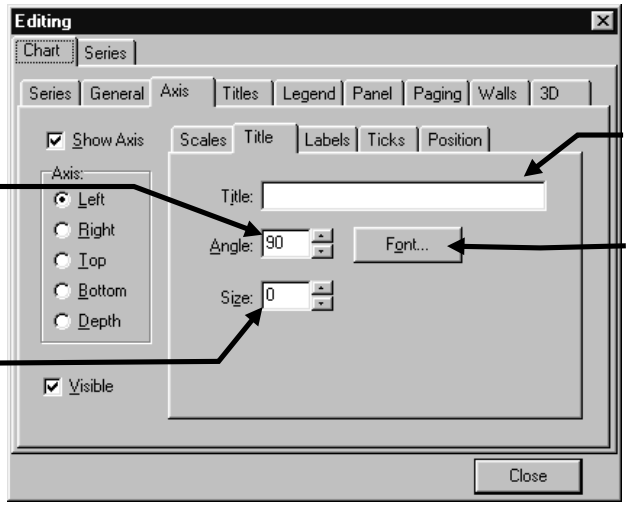
Sets the number of steps of the values displayed in the chart axis labels.

Enables or disables the inverted chart display.

Enables or disables the logarithmic chart display.

- [Title] sub-panel

Used to set the title of the coordinate axis.



Sets the angle of displaying the coordinate axis title.

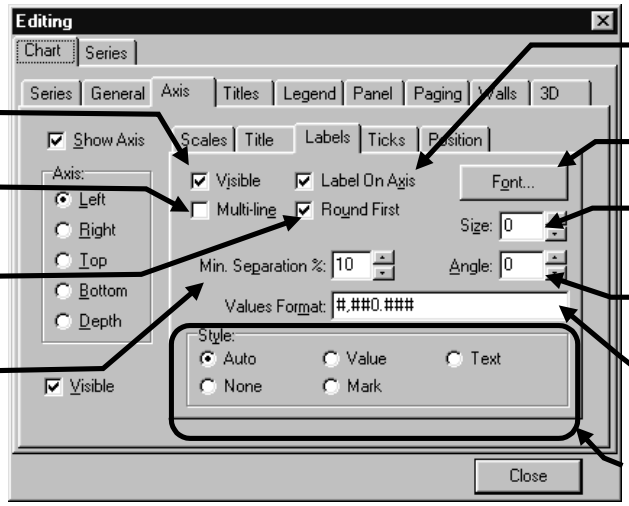
Sets the angle of displaying the coordinate axis title.

Sets the title of the coordinate axis.

Sets the title character font.

- [Labels] sub-panel

Used to set the label of the coordinate axis.



Enables or disables the label display.

Not used with the present application.

Enables or disables the scale display according to the maximum and minimum values in the chart.

Sets the distance between labels.

Enables or disables the label display on a point of crossing with another axis.

Sets the label character font.

Sets the distance from the title to the label display position.

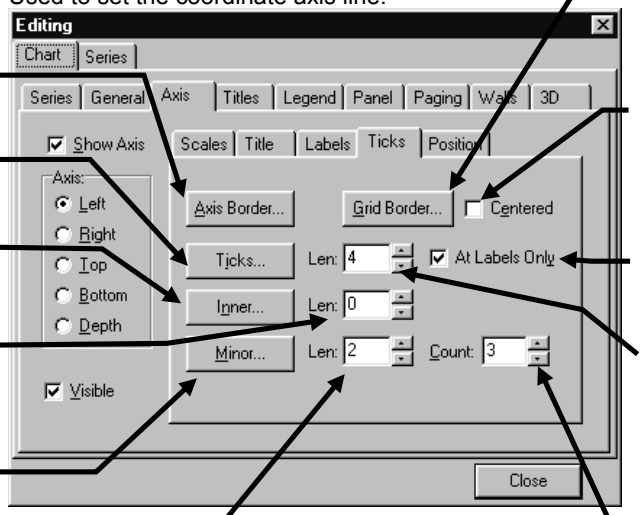
Sets the angle of label display.

Sets the label display format.

Sets the label type. Select [Auto] or [None].

- [Ticks] sub-panel

Used to set the coordinate axis line.



Sets the type, color and width of the coordinate axis.

Sets the type, color and width of the scale line on the label side of the coordinate axis.

Sets the type, color and width of the scale line on the chart side of the coordinate axis.

Sets the length of the scale line from the coordinate axis to the chart side.

Sets the type, color and width of the scale line between labels.

Sets the length from the coordinate axis of the scale line between labels to that on the label side.

Sets the type, color and width of the line along the label.

Enables or disables the display of coordinate axis scales in the intermediate position between labels.

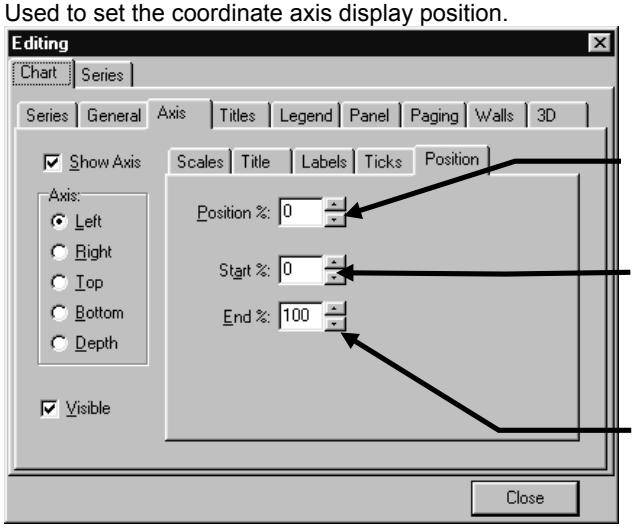
Enables or disables the display of scale line in the positions where labels are displayed.

Sets the length of the scale line from the coordinate axis to the label.

Sets the number of division between labels.

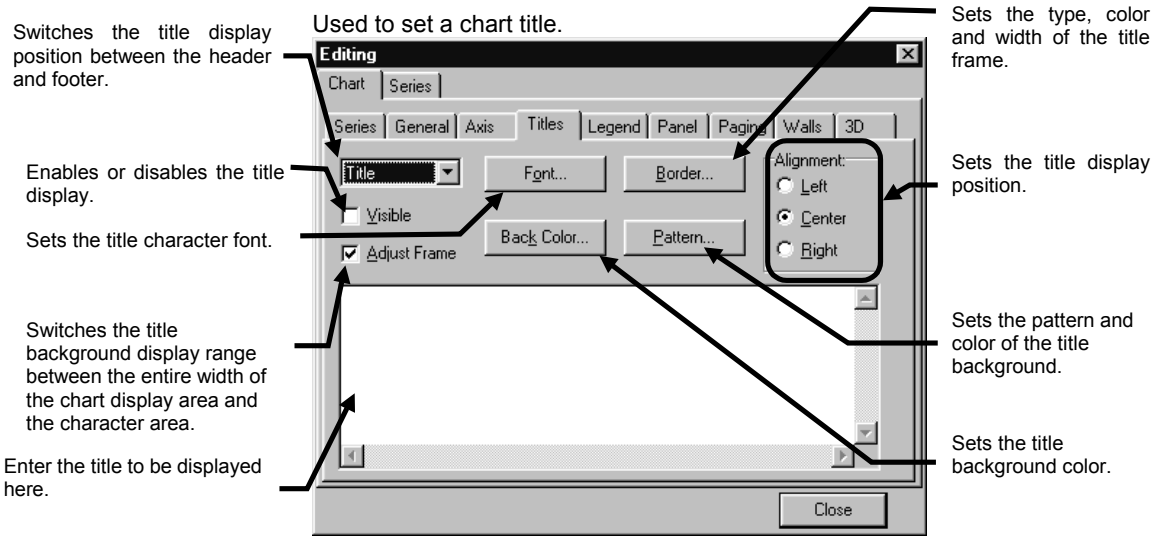
- [Position] sub-panel

Used to set the coordinate axis display position.



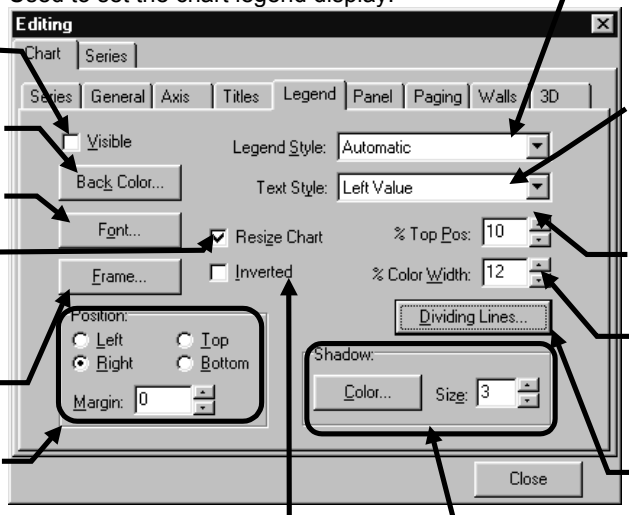
- [Titles] sub-panel

Used to set a chart title.



- [Legend] sub-panel

Used to set the chart legend display.



Enables or disables the legend display.

Sets the legend background color.

Sets the legend character font.

Sets whether or not the chart is resized according to the legend display.

Sets the type, color and width of the legend frame line.

Sets the legend display position and the margin between the chart display area and legend display area.

Sets the items to be displayed in the legend. Usually set [Automatic].

Sets the legend display style. This setting is effective when the numerical value display is selected in the [Legend Style] pull-down menu.

Fine-adjusts the positioning of the legend display from the top edge.

Sets the width of the lines displayed in the legend column.

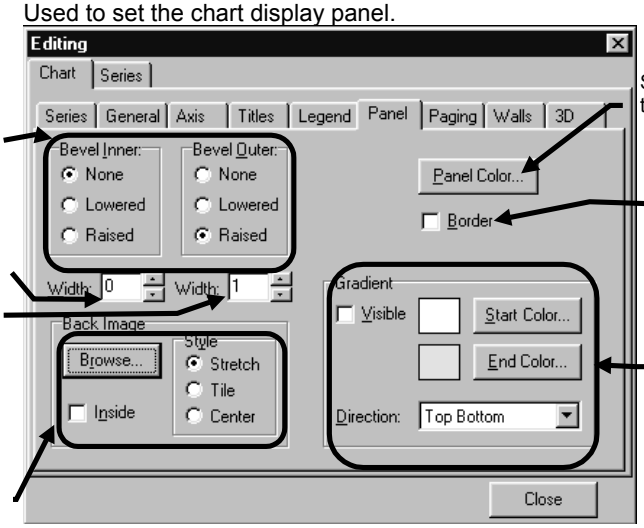
Sets the type, color and width of the lines dividing the legend items.

Enables or disables the inversion of the display order of legend items.

Sets the color and width of the shadow area of the shadowed display of legend area.

- [Panel] sub-panel

Used to set the chart display panel.



Set the format of the borders of the chart display area. [Bevel Inner] sets the inner borders of the chart display area and [Bevel Outer] sets the outer borders of the chart display area.

Sets the width between the inner and outer borders.

Sets the width of the inner and outer borders.

Specifies the background image of the chart display area, sets whether it is displayed inside the chart or in the entire chart display area, and sets its display position.

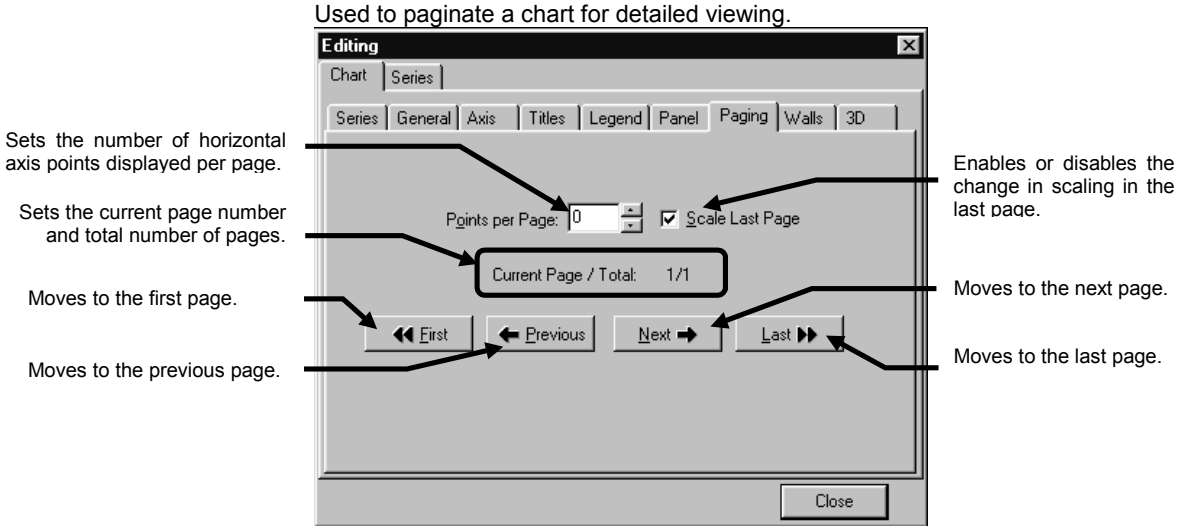
Sets the background color of the chart display area.

Enables or disables the display outside the outer frame of the chart display area.

Sets whether or not the chart display area background is displayed with gradations, and sets the colors and type of the background.

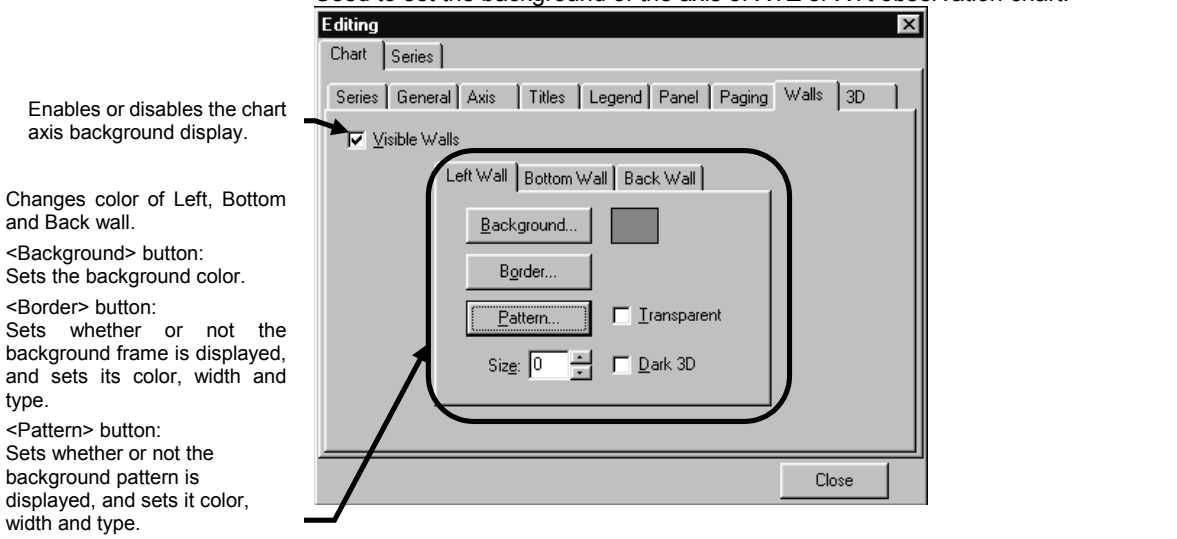
- [Paging] sub-panel

Used to paginate a chart for detailed viewing.



- [Walls] sub-panel

Used to set the background of the axis of XYZ or XYt observation chart.



Changes color of Left, Bottom and Back wall.

<Background> button:
Sets the background color.

<Border> button:
Sets whether or not the background frame is displayed, and sets its color, width and type.

<Pattern> button:
Sets whether or not the background pattern is displayed, and sets it color, width and type.

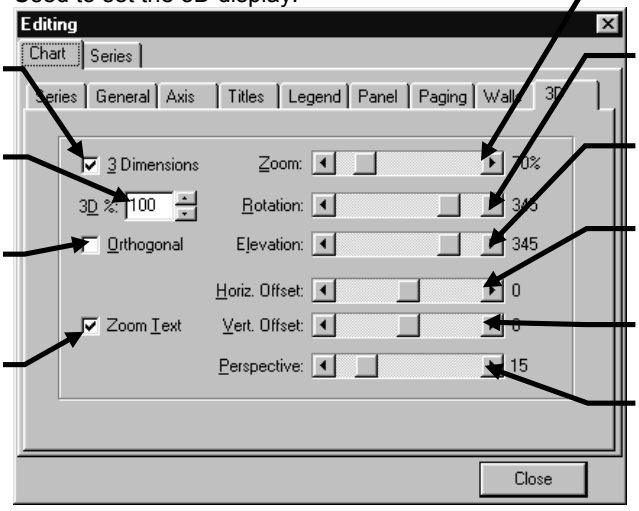
[Transparent] check box:
Enables or disables the transparent background display.

[Size] text box:
Sets the background thickness.

[Dark 3D] check box:
Enables or disables the shadowed, 3D display.
This check box can be selected when the background thickness is specified.

- [3D] sub-panel

Used to set the 3D display.



Switches between the 3D and 2D chart display.

Sets the 3D chart display effect by varying the angle.

Enables or disables the 3D display in the depth-wise direction.

Enables or disables the zooming display of character strings in the chart, such as label characters, together with the chart display.

Change the zoom ratio.

Rotates the chart in the horizontal direction with respect to the bottom side.

Rotates the chart in the vertical direction with respect to the bottom side.

Moves the chart display position in the horizontal direction.

Moves the chart display position in the vertical direction.

Distorts the chart to change the display effect.

2-14-2 [Series] Panel

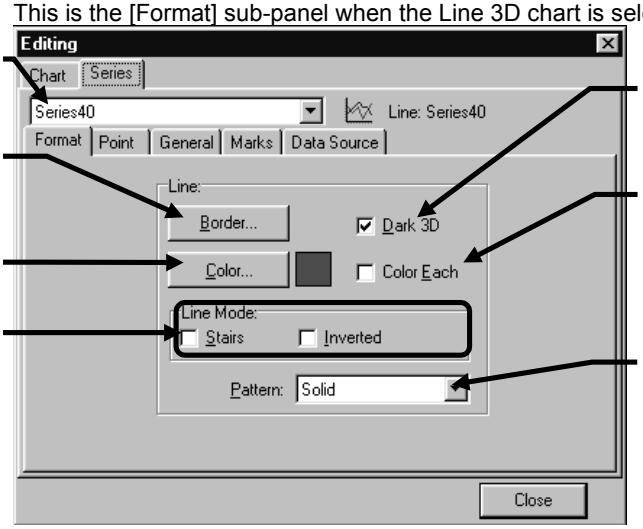
- [Format] sub-panel

  Used to set the display method of a series of images.

  The set items are variable depending on the type of the chart.

  The following descriptions take a Line 3D chart and Point 3D chart as examples.

This is the [Format] sub-panel when the Line 3D chart is selected.



Select the chart series to be set in the [Series] panel.

Sets whether or not the outer frame of the chart is displayed, and sets the color, width and type of the frame.

Sets the chart color.

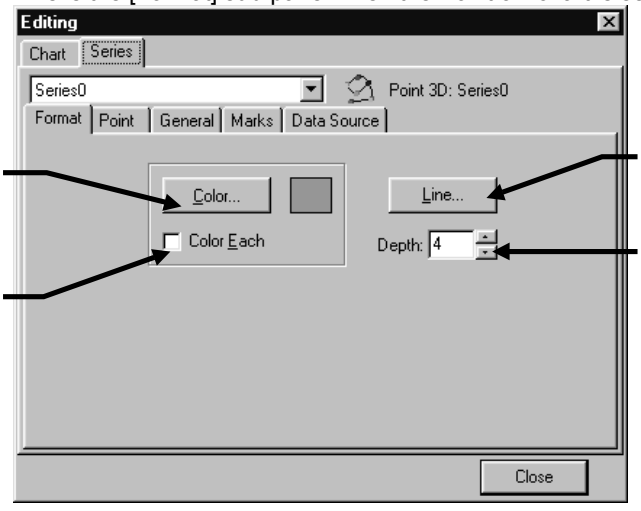
Enables or disables the stairstep type chart display.

Enables or disables the shadowed 3D display.

Enables or disables the coloring per coordinate axis.

Sets the chart pattern.

This is the [Format] sub-panel when the Point 3D chart is selected.



Sets the chart color.

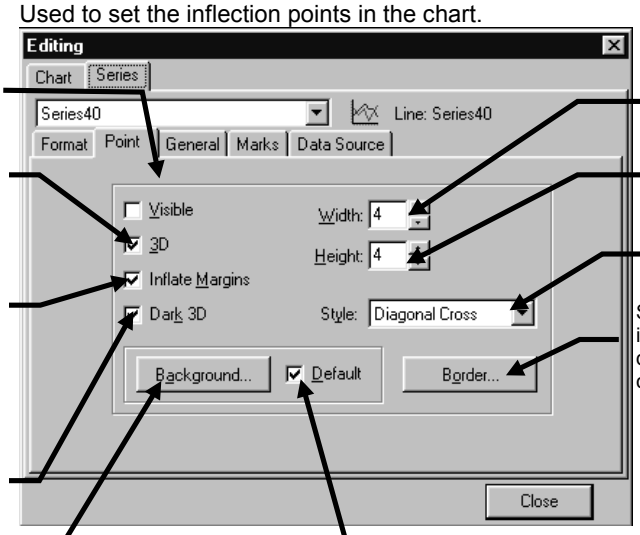
Enables or disables the display varying the color at every point in the chart.

Sets whether or not chart lines are displayed, and sets the color, width and type.

Sets the depth of points in the chart.

- [Points] sub-panel

Used to set the inflection points in the chart.



Enables or disables the inflection point display.

Enables or disables the 3D inflection point display.

Selects whether the ends of the inflection points are aligned with the coordinate axis (when checked) or the centers of the inflection points are aligned with the coordinate axis (when unchecked).

Enables or disables the dark shadowed 3D display of inflection points.

Sets the inflection point background color.

Sets the width of inflection points.

Sets the height of inflection points.

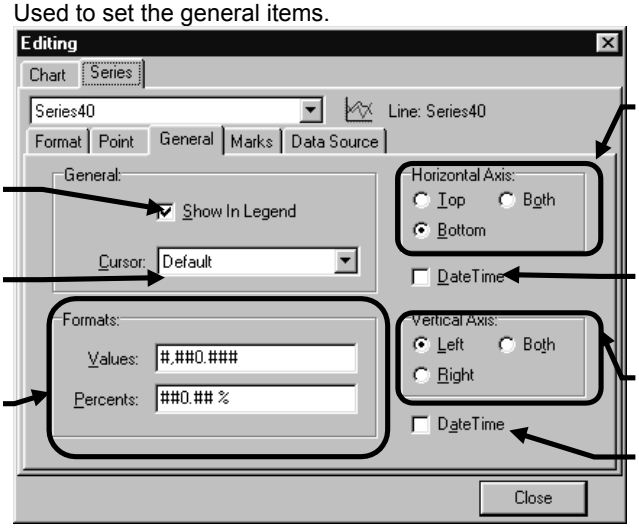
Sets the type of inflection points.

Sets whether or not the inflection point borders are displayed, and sets the color, width and type.

Resets the background color to the default color.

- [General] panel

Used to set the general items.



Enables or disables the chart display inside the legend.

Sets the shape that the cursor changes when it is placed on the chart.

Sets the display formats when the label display is set to the numerical value display or percent display.

Selects whether the labels are displayed on or below the chart.

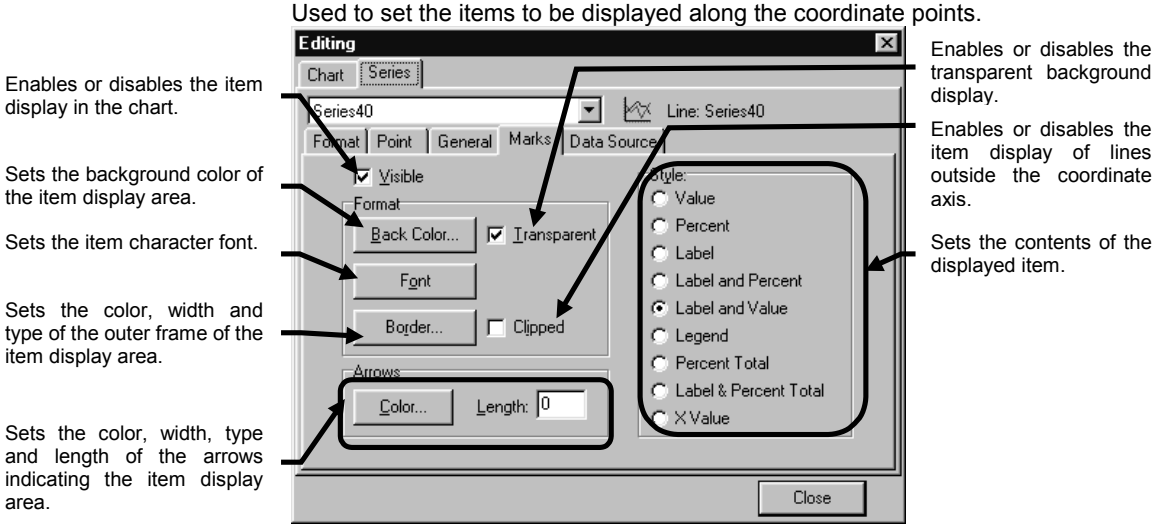
Usually leave this box unchecked.

Selects whether the labels are displayed to the left or right of the chart.

Usually leave this box unchecked.

- [Marks] sub-panel

Used to set the items to be displayed along the coordinate points.



- [Data Source] sub-panel

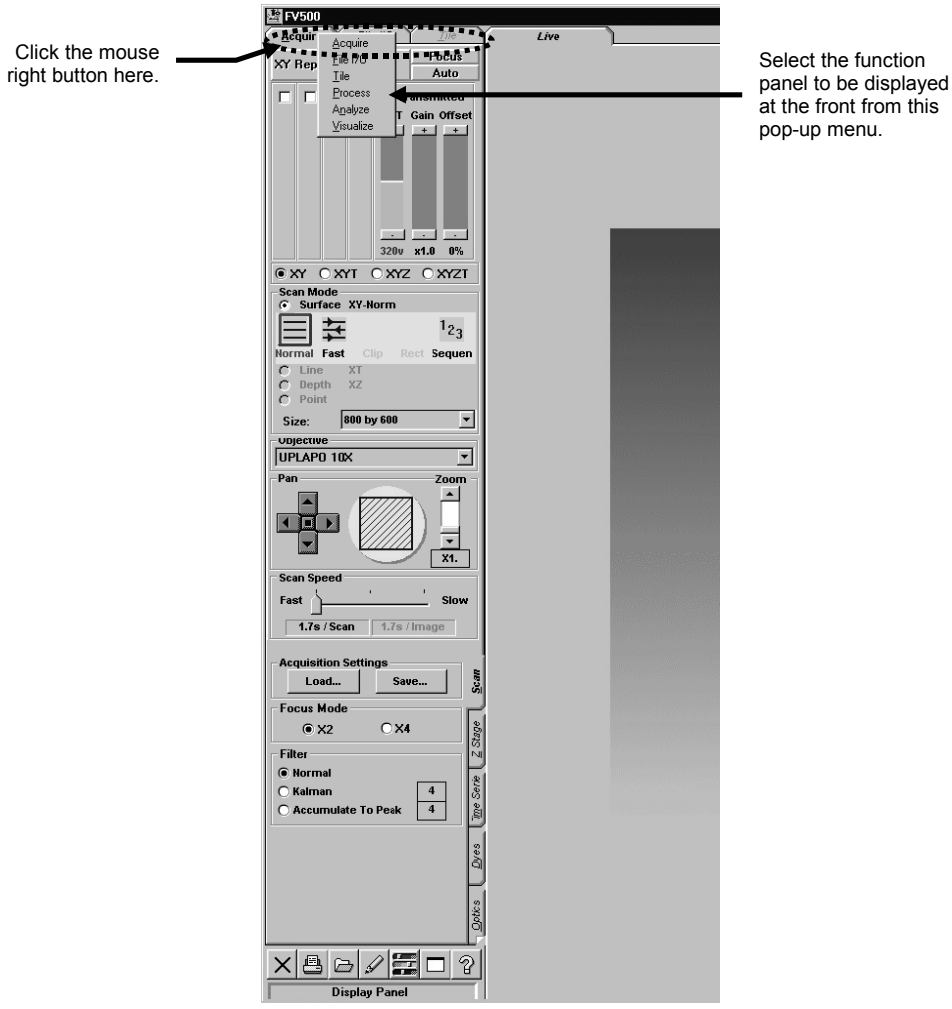
Do not change the settings here but leave them in the initial condition.

2-15 Pop-up Menus

Selection of function panels and display panels and other frequently-used FLUOVIEW functions (full-screen display, printer output, image save, LUT setting, comment setting) can be controlled by clicking the right button of the mouse, without selecting specific page tabs or buttons.

●Pop-up menu of function panel

When the right button of mouse is clicked on the page tab of a function panel, a pop-up menu appears to allow selection of the function panel to be displayed at the front.



●Pop-up menu of display panel

When the right button of mouse is clicked on the page tab of a [Display] panel, a pop-up menu appears to allow selection of the [Display] panel to be displayed at the front.

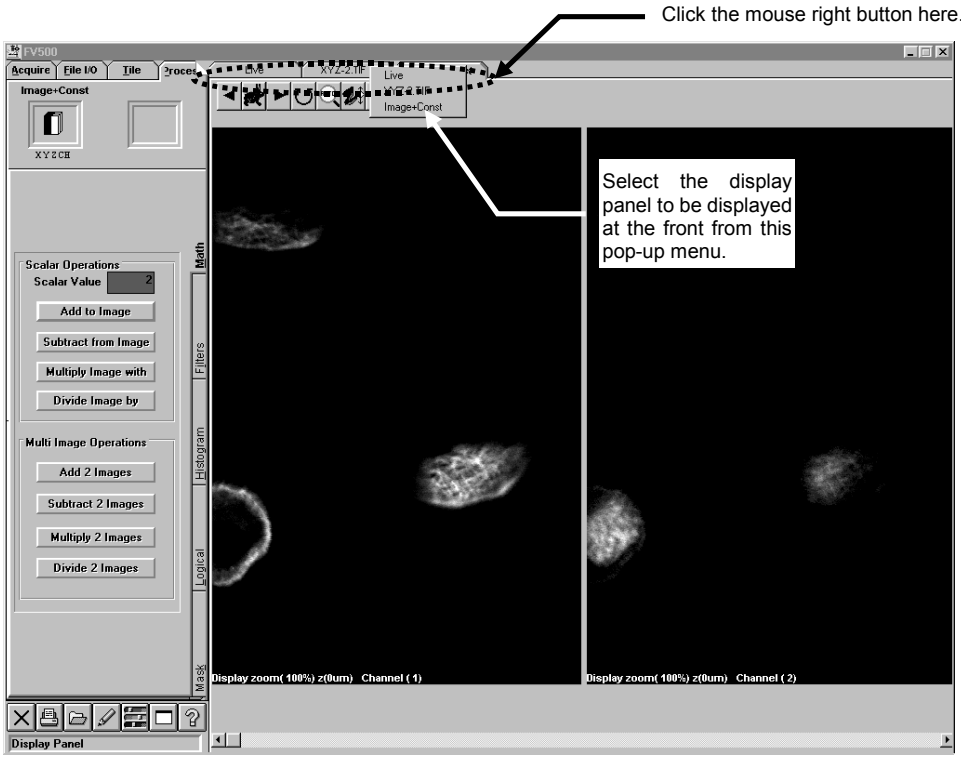


Fig. 2-128 [Display] Panel Showing Pop-up Menu

●Pop-up menu of comment

When the right button of mouse is clicked on an comment in image, a pop-up menu appears to allow editing or deleting the specified comment.

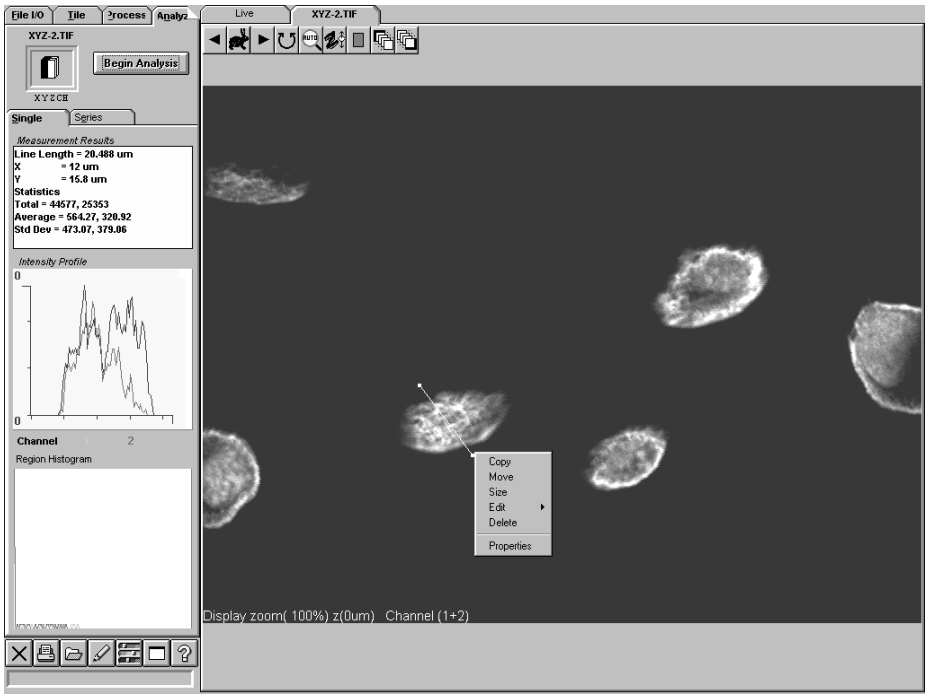
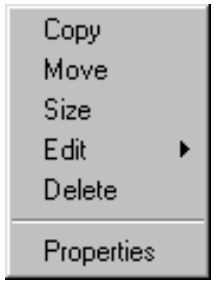


Fig. 2-129 Image Comment with Pop-up Menu



Copies the comment.

Moves the comment.

Resizes the comment.

Edits the selected arrow or scale.

Deletes the comment.

Edits the comment color and font.

●Pop-up menu of image

When the right button of mouse is clicked on the image, a pop-up menu appears to allow selection of image operations (full-screen display, printer output, image save, LUT setting, number of image divisions, comment editing).

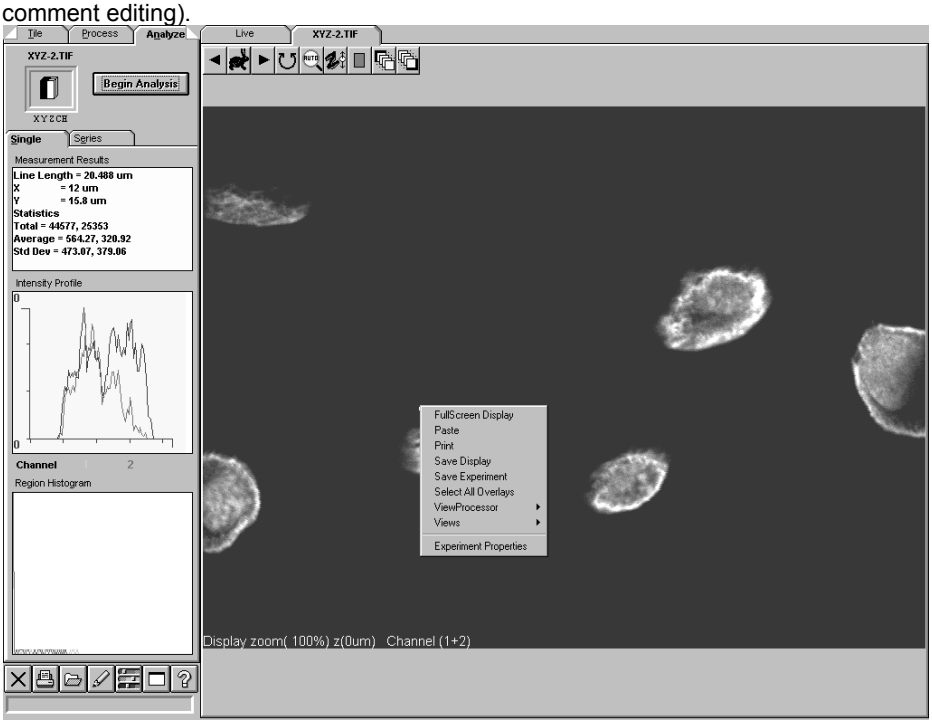


Fig. 2-130 [Display] Panel Showing Pop-up Menu

FullScreen Display	Displays the image full-screen.
Paste	Pasts the comment copied on the image.
Print	Outputs the image at the printer.
Save Display	Saves the image as a single image.
Save Experiment	Saves the image as a series of images.
Select All Overlays	Selects all the comments overlaid on the image.
ViewProcessor ▶	Edits the image LUT.
Views ▶	Sets the number of image divisions when displaying more than one image.
Experiment Properties	Edits the image comment.

●Pop-up menu of the [Experiments in Memory] dialog box

When the right button of mouse is clicked in the frame for opening an image file in the [File I/O], [Tile] or [Process] panel, a pop-up menu appears. This makes it possible to select the file to be opened when performing tile display, inter-image operations, image channel merger/extraction, etc.

Click the mouse right button here.

The pop-up menu appears showing the file names that can be selected.

Select the file name to be processed.

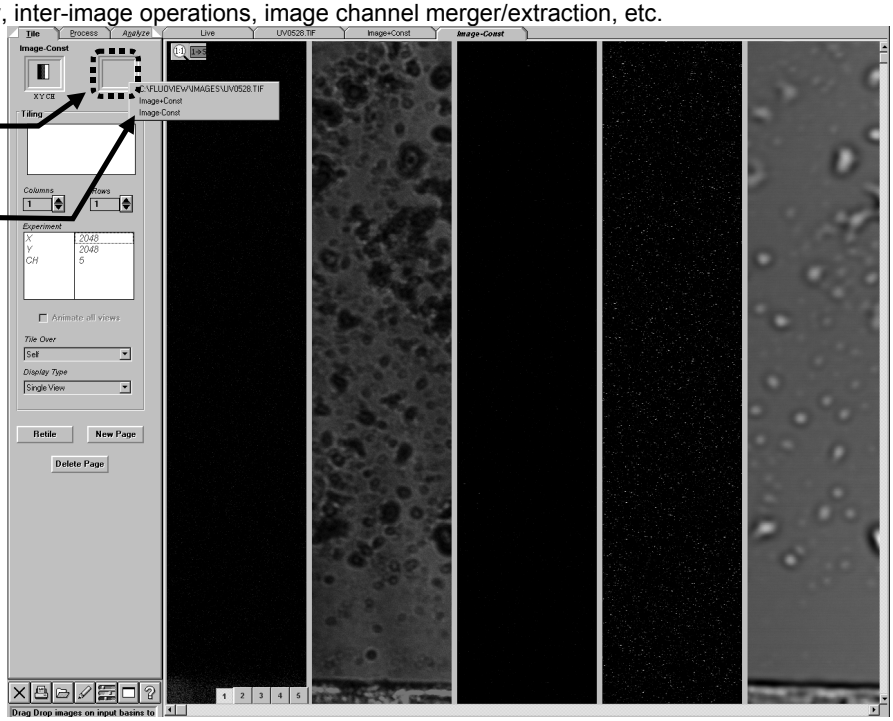


Fig. 2-131 Function Panel Showing Pop-up Menu

Appendix A List of Tools

Appendix A-1 List of Tools

Appendix A-1-1 Toolbar

The horizontal array of buttons at the bottom left of the panel form the toolbar. The most frequently-used FLUOVIEW functions are gathered here.



<Help> button

Displays on-line help.

Allows the function and operation descriptions to be referenced while operating the application.

<Full Screen> button

Displays the image full screen.
Allows only the image to be displayed by erasing all other image components such as the toolbar, panels and status bar.

<LUT> button

Allows the simulated colors to be changed or created originally by editing the LUT (Look Up Table).

<Annotate> button

Allows the analysis area to be specified or comment to be drawn in the image.

<Experiment List> button

Allows the processing target image to be selected or deselected.

<Print> button

Outputs the image at the printer.

<Exit> button

Exits from FLUOVIEW.

Appendix A-1-2 Tools at the Top of Display Panel

These refer to the horizontal array of buttons at the top of the display panel. The functions frequently used with images are gathered here.

<Set end position> button

When successive display or frame-by-frame display is required, set the image with which the display should end. ("n-1", assuming that the number of images is n, is set when this button is not used.) This button is also used to set the image for ending a processing operation such as image save.

<Set start position> button

When successive display or frame-by-frame display is required, set the image with which the display should start. ("0" is set when this button is not used.) This button is also used to set the image for starting a processing operation such as image save.

<Display switching> button

Used to display more than one channel simultaneously.

<Z/T series switch> button

When an image is composed of multiple image slices, this button switches between the display according to multiple sections and that according to the progress of time. This button is also used to display an extended image.

<Magnify/reduce> button

Magnifies or reduces the image. Magnification or reduction by 3:1 or 1:3 the original image size is possible.



<Bound>/<Loop> buttons

Set the repetition direction of the successive display or frame-by-frame display.

<Display> button

Press to start display of n-slices of image from the start position to the end position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.

Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

<Display speed switch> button

Set the speed of successive display.

<Display> button

Press to start display of n-slices of image from the end position to the start position sequentially (successive display) or one by one (frame-by-frame display) or to stop successive display.

Holding the button displays image slices in successive display and clicking it displays image slices by advancing frame by frame.

Appendix B List of Hot Keys

Frequently-used FLUOVIEW functions (scanning, panel switching) can be controlled from the keyboard without using the mouse.

- Image acquisition-related keys

Image acquisition channel selection

Key	Target Operation
Cttrl + 1	Switches the status of the Ch1 check box (Ch1 scanned/not scanned).
Cttrl + 2	Switches the status of the Ch2 check box (Ch2 scanned/not scanned).
Cttrl + 3	Switches the status of the Ch3 check box (Ch3 scanned/not scanned).
Cttrl + 4	Switches the status of the Ch4 check box (Ch4 scanned/not scanned).
Cttrl + 5	Switches the status of the Ch5 check box (Ch5 scanned/not scanned).

PMT voltage/gain/offset adjustments

Key	Target Operation
←	Enables and makes variable the LED slider on the left.
→	Enables and makes variable the LED slider on the right.
↓	Decreases the value of an enabled LED slider. (Fine adjustment)
↑	Decreases the value of an enabled LED slider. (Coarse adjustment)
Shift + ↓	Increases the value of an enabled LED slider. (Fine adjustment)
Shift + ↑	Increases the value of an enabled LED slider. (Coarse adjustment)

NOTE **An LED slider is enabled and variable when its setting values are displayed in red.**

Image acquisition (scanning)

Key	Target Operation
F1	Displays the on-line help screen.
F2	Performs repeated scanning. [XY Repeat]
F3	Starts scanning according to the current scan mode. [Scan Once]
F4	Performs focus scanning. [Focus]
F6	Acquires another image after completing series image acquisition. [Append Next]
F7	Completes image acquisition after completing series image acquisition. [Series Done]
Spave	Stops scanning. [Stop Scan]

Scanning speed and area setting

Key	Target Operation
Ctrl + →	Decreases the scan speed.
Ctrl + ←	Increases the scan speed.
Ctrl + ↓	Decreases the zoom ratio.
Ctrl + ↑	Increases the zoom ratio.

Z stage setting

Key	Target Operation
Page Up	Moves the Z stage up. (Fine adjustment)
Shift + Page Up	Moves the Z stage up. (Coarse adjustment)
Page Down	Moves the Z stage down. (Fine adjustment)
Shift + Page Down	Moves the Z stage down. (Coarse adjustment)
Ctrl + Page Up	Sets the Z stage to the Stop Z position.
Ctrl + Page Down	Sets the Z stage to the Start Z position.
Insert	Switches the [Locked] check box (motor excitation) status.
Ctrl + Delete	Cancels the Start and Stop settings. <Set Zero> Z motor: The current position becomes 0.0.

Key	Target Operation
	Piezo: Returned to the 0.0 position.
Home	Moves the stage till the Stop Z position.
End	Moves the stage till the Start Z position.

●Transmitted illumination lamp ON/OFF keys

Key	Target Operation
Ctrl + L	Switches the transmitted illumination ON/OFF. <Trans. Lamp>

- Image save key

Key	Target Operation
Ctrl + S	Save the image in the [Display] panel being displayed as series images. <SAVE>

- Panel select keys

Main panel

Key	Target Operation
Alt + A	Selects the [Acquire] panel.
Alt + F	Selects the [File I/O] panel.
Alt + T	Selects the [Tile] panel.
Alt + P	Selects the [Process] panel.
Alt + N	Selects the [Analysis] panel.
Alt + V	Selects the [Visualize] panel.

[Acquire] sub-panels

Key	Target Operation
Alt + S	Selects the [Scan] sub-panel.
Alt + Z	Selects the [Z Stage] sub-panel.
Alt + M	Selects the [Time Series] sub-panel.
Alt + D	Selects the [Dyes] sub-panel.
Alt + G	Selects the [Config] sub-panel.

[Process] sub-panels

Key	Target Operation
Alt + M	Selects the [Main] sub-panel.
Alt + I	Selects the [Filter] sub-panel.
Alt + H	Selects the [Histogram] sub-panel.
Alt + L	Selects the [Logical] sub-panel.

[Analysis] sub-panels

Key	Target Operation
Alt + S	Selects the [Single] sub-panel.
Alt + E	Selects the [Series] sub-panel.

[Visualize] sub-panels

Key	Target Operation
Alt + O	Selects the [Orientations] sub-panel.
Alt + R	Selects the [Other options] sub-panel.

Appendix C Glossary

A

AOTF
AOTF represents Acoustic Optical Tunable Filter.
AOTF is the sound optical element having optical anisotropy. When a sound wave is spread in the element, the element behaves as a phase grid and only the light of the wavelength corresponding to the wavelength of a sound wave is diffracted in the specific direction. Therefore, it is possible to control selection of laser wavelength and adjustment of laser intensity at high speed.

Active
Status of being selected or executable. An active window can be distinguished from other windows by the color of the title bar.

Application
Same as “software”. Among software, refers to the software used directly by the users.

B

Backlash
The quantity of play (or loosening between gear teeth and part) which is produced when the stage is moved up or down by the Z-motor.

Button
→Command button.

C

Check box
A small square which can be either checked with ✕ or cleared. The check box indicate an item which can be enabled or disabled. The item is enabled when it is checked with X.

Clear
Action of removing check mark ✕ from a check box to disable the item. To clear a check box checked with ✕, click the check box.

Click
Action of pressing then releasing the button of the mouse.

Clipboard
The place which relays data when an operation such as “Copy”, “Cut” and “Paste” is executed.

Command button
A figure in the shape of button in the window. Clicking a command button with the mouse allows the function indicated on the button to be executed.

Confocal
Signifies the possibility of obtaining data on the plane where the irradiated laser beam is focused.

Contrast
Variation (change) between the brightest and darkest areas in an image.

Control menu
The menu displayed when the control menu box at the left end of the title bar of a window is clicked. When the window is minimized, the control menu can be displayed by clicking the icon. The control menu contains commands for controlling the window.

Control menu box
The square button at the left end of the title bar of a window. Clicking this box opens the control menu.

Copy
Action of placing selected data in the clipboard so that the data can be placed in other place later.

Cursor
Blinking | which indicates the area where a character can be input. When a keyboard key is pressed, the character is entered in the position of the cursor.

D

Dialog box
Some functions require fine settings so that they can be executed, and some functions require the confirmation of settings before being executed. The dialog box is a sub-window displayed in such cases.

Directory
Hierarchical classification of the space inside a disk so that the files can be arranged in a significant manner according to their categories.

Disk drive
The storage device storing the files. Some disk drives such as the hard disk drive and floppy disk drive are capable of both input and output, and some such as the CD-ROM drive is designed for read only.

Dot
→Pixel.

Double-click
Action of pressing and releasing the mouse button quickly twice, without moving the moue.

Drag
Action of placing the mouse pointer on the target function, pressing the mouse button, moving the mouse while keeping the mouse button pressed, and releasing the mouse button at the destination position.

Drive
→Disk drive.

Drive name
Character such as “A” and “C”, assigned to each drive. → Disk drive.

E

Extended focus
View of an XYZ image obtained by projection in the Z-direction.

Extension
Up to 3 characters after a period, which are attached at the end of a file name. The extension usually represents the type of the file or directory.

F

File
Group of information which is named and saved in a disk

G

Gain
This function brightens the image by the ratio set at the time of image acquisition. Use Gain when a bright image cannot be obtained even by setting PMT Voltage to 800 V.

Group
Refers to the applications registered in the program manager. When a group is opened on the program manager, more than one icon is displayed in the window. When the group is closed, the group becomes an icon of the window.

I

Icon
An icon is a small figure with characters below it. The icon indicates the status in which the window is closed (or minimized).

Iconize
This refers to turn a window into an icon display by using the iconize button of the [Iconize] command in the control menu. The application continues to run even after it has been turned into an icon. Selecting an icon returns it to the active application.

Intensity
Brightness of each pixel in an image.

Items displayed in pale color
The menu commands and buttons which cannot be used are displayed in less visible way, i.e. in a pale color or gray.

K

Keyboard input
Action of inputting an alphanumeric character from the keyboard of the computer.

L

LUT(LookUpTable)
The image acquired by observation (input) has 12 bits of brightness data per pixel. Meanwhile, the brightness data which can actually be displayed (output) consists of 8 bits per pixel for each of R, G and B. The LUT is the tabulation of this relationship between the input and output.

M

Macro
Record of a series of operations. When a macro is executed, the series of operations defined for it are executed.

Maximize button
The button showing an upward triangle at the right of the title bar. Clicking this button displays the window full screen. The same operation is also available using the [Maximize] command in the control menu.

Mouse
A device which was named because it looks like a mouse. It is used to give instructions in the window.

Mouse pointer
When the mouse is moved on the desk, arrow ↖ moves in the display along the movement of the mouse. This "↖" is called the mouse pointer.

O

Offset
This function darkens the image by the ratio set at the time of image acquisition.
(Offset should be used before using Gain.)

On-line help
Manual displayed on the screen, that is built into software.

Option button
Small circular button inside a rectangular frame in a window. Only one option button (item) can be selected from the items enclosed in the frame.

P

Panel
The large rectangle with a page tab in a window. The panel is provided on a per-function basis, and clicking the page tab displays the panel of the selected function.

Paste
Action of placing the data in the clipboard in an application.

Piezo-
Abbreviation of piezoelectric, which is the phenomenon of generating electricity when a force is applied. This phenomenon is utilized in electric spark generators for use with gas appliances, etc.

Pixel
The minimum graphic unit of screen display. Also referred to as the dot.

PMT Voltage
Increasing this setting increases the sensitivity.
When a bright image cannot be obtained even by setting PMT Voltage to 800 V, do not vary PMT Voltage any more but increase Gain. This usually provides a better effect than increasing PMT Voltage above 800 V.

R

Resolution
Number of dots composing the image on a screen or printer. When the number of dots is increased, i.e. when the resolution is increased, the gradation can be displayed in more details.

Reversed display
Display method which indicates that an item or character string is selected and has become the target of the user's next operation. The reversed displayed characters are shown in a different color from other characters.

S

Scroll
Action of moving text or a picture up or down in order to view the other part of information than the information which can be displayed at once in the window.

Scroll bar
The bar displayed at the bottom or right of a window containing more information than can be displayed at once. The scroll bar has a knob and two arrow buttons. Dragging the knob scrolls the information directly and clicking one of the arrow buttons scrolls the information line by line. Holding an arrow button scrolls the information continuously.

Simulated colors
Colors used to display the image data acquired by observation on a display. Original simulated colors can be created by editing the LUT.

Status bar
The line showing information at the bottom of a window. It shows the information on the operation or the description of the function selected with the mouse pointer.

T

Text
A file expressed with the ASCII codes such as characters and numerals, and with some control codes such as the line feed code. This format is referred to as the text format. The text is usually input from the keyboard.

Text box
A box in the window that accepts the input of character strings. Clicking a text box displays blinking |. This "|" indicates the position where the input character is inserted.

Title bar
The horizontal bar at the top of the window, that shows the title of the window or dialog box.

Toolbar
The toolbar provides frequently used functions in the form of buttons. It can be used any time during execution of any function.

W

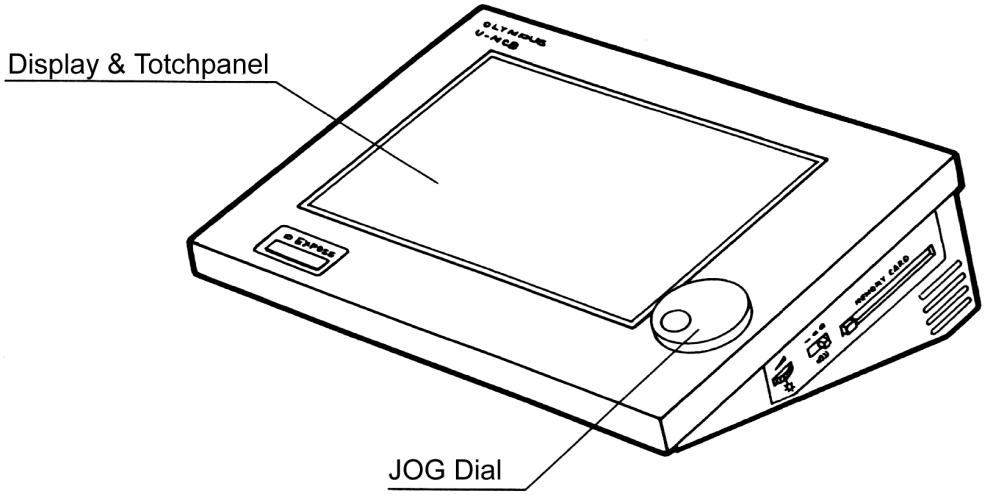
Window
A large rectangle with a title. A window can be opened or closed.

Appendix D U-MCB Setting

Please set U-MCB before using this system.

NOTE

Switch the display for setting of the multi control box to MAIN to operate it through the FLUOVIEW software after the setting has been completed.



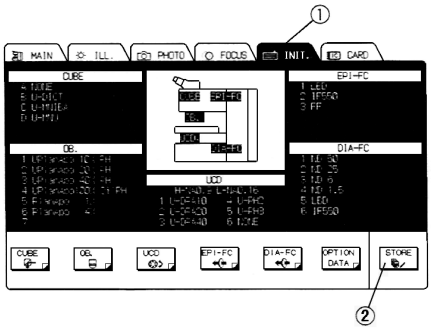


Fig. D-1

1. Press the INIT.(1) mode selector button. to display the environment data input screen.(Fig.D-1)

TIP

Setting of unattached, related module units may also be performed on this screen.

NOTE

During setting of the environment data. the controls of the hand switch(U-HS,U-HSTR) and the AX70 microscope frame (e.g.lamp ON/OF button) will be inoperative.

NOTE

Always remember to press the STORE button (2) after setting all the INIT.data.(Fig.D-1)

If the STORE button is not pressed, all the already set data will be erased when another screen (MAIN, PHOTO ,etc.) is selected with the mode selector buttons.

However, the STORE operation is not required after setting the time only.

Appendix D-1 Mirror Cube Data Input(CUBE)

Please register the fluorescence cubes for fluorescence observations.

NOTE **Please do not do full registration. Leave at least one position "NONE" for use in LSM observation.**

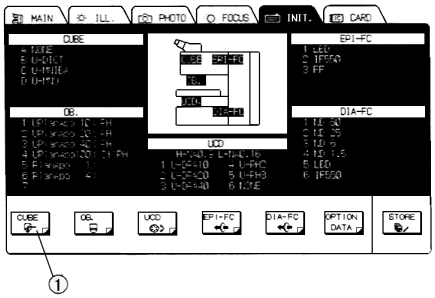


Fig. D-2

1. When the CUBE button on the INIT. input screen is pressed, the CUBE DATA SET screen will appear as shown in Fig.D-2.
   The data field (1) shows the currently entered values.

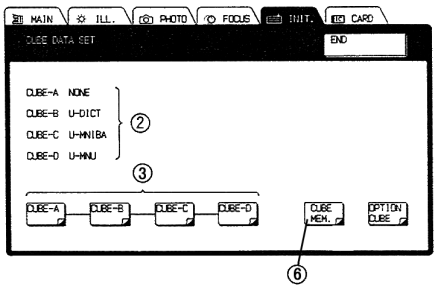


Fig. D-3

2. When the cube button (2) corresponding to the cube to be engaged is pressed, the screen shown in Fig.D-4 will appear and the specified cube will be engaged into the light path.
3. Rotating the JOG dial, select the name of the cube engaged into the light path.
4. When END (5) is pressed, the first cube name is specified and the previous screen returns.
5. To enter all the required cubes, repeat the above procedure.
6. The currently displayed values can be stored as one set (4 cubes). Stored data can then be loaded, stored, and confirmed for future use.
   1) To display the screen shown in Fig.D-3, press CUBE MEM. (6)(Fig.D-3)
   2) Press STORE(7). Using the JOG dial, select the MEMORY number in which to store.
   3) When EXECUTE(8) is pressed, the confirmation screen will appear. If correct, press ENTER. If something is wrong, press CANCEL.
   4) When END is pressed, storage is performed, and the previous screen will return.

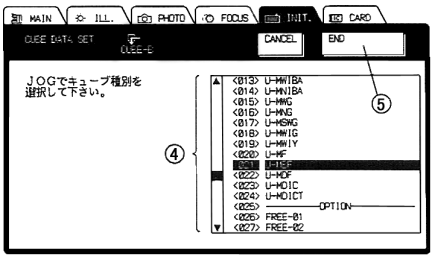


Fig. D-4

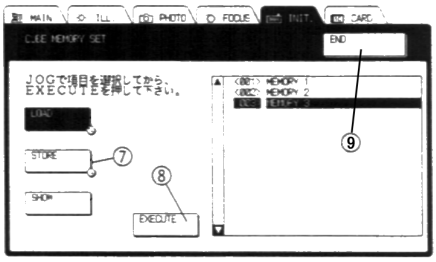


Fig. D-5

Appendix D-2 Objective Data Input(OB.)

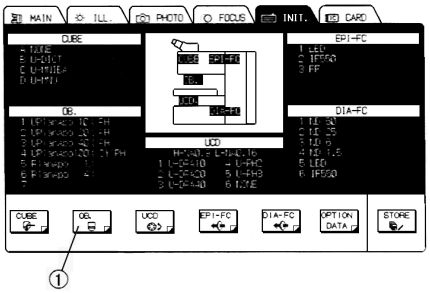


Fig. D-6

1. To display the OB. LENS DATA SET screen (Fig.D-7), press OB. (1) on the INIT. input screen (Fig.D-6).

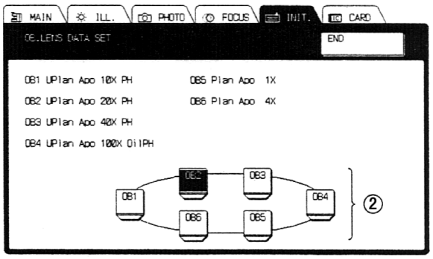


Fig. D-7

2. When the objective number (2) to be entered is pressed, the screen shown in FIg.D-8 will appear. The revolving nosepiece will rotate and the objective at the specified nosepiece position will be engaged.

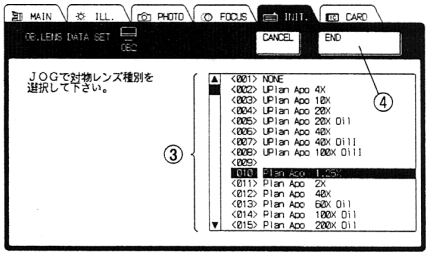


Fig. D-8

3. Rotating the JOG dial, select the designation (3) of the objective engaged into the light path.
4. When END(4) is pressed, the first objective designation is entered and the previous screen will return.
5. to enter the data of other objectives, repeat the above procedure.

The following table shows the objectives

Objective
PLAPO 40X
PLAPO 60XO
PLAPO 100XO
PLAPO 40XWLSM
PLAPO 60XWLSM
PLAPO 60XOLSM
UPLAPO 10X
UPLAPO 20X
UPLAPO 20XO
UPLAPO 40X
UPLAPO 40XOI
UPLAPO 60X
UPLAPO 60XW
UPLAPO 60XWPSF
UPLAPO 100XOI
UPLFL10X
UPLFL20X
UPLFL40X
UPLFL60XOI
UPLFL100XO
UPLFL100XOI
UMPLFL10XW
UMPLFL20XW
LUMPLFL40XW
LUMPLFL60XW

Appendix D-3 Transmitted light DIC prism Setting (UCD)

Please register the DIC prism is suitable for a objective.

NOTE **In the case that you do not have the suitable prism for a objective lens, please register the prism temporarily.**

NOTE **Leave at least one position "NONE" for use in microscopic observation using the FV500 system.**

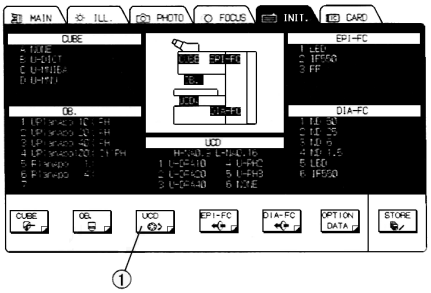


Fig. D-9

1. To display the UCD DATA SET screen (Fig.D-10), press UCD(1) on the INIT. input screen(Fig.D-9).

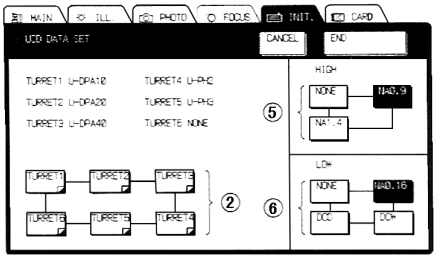


Fig. D-10

2. When the TURRET number (2) to be entered is pressed, the screen shown in Fig.D-11 will appear. The turret will rotate and the specified turret aperture will be placed at the position of the turret's optical element mounting hole.

TIP When setting and specifying the turret aperture, the optical element is placed at the position of the turret's optical element is placed in the optical axis.

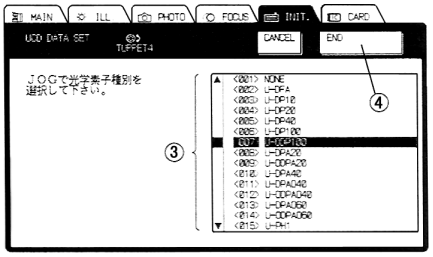


Fig. D-11

3. Using the JOG dial, select the designation of the optical element (3) positioned at the turret's optical element mounting hole.
4. When END(4) is pressed, the first optical element is entered and the previous screen will return.
5. To enter the data of other optical elements, repeat the above procedure.
6. Select either the HIGH (5) or LOW (6) magnification top lens type.(Fig.D-10)

NOTE **For details on using either high or low magnification top lens, refer to the instruction manual for the condenser.**

The following table shows which DIC prism is suitable for a objective lens.

Objective	Transmitted light DIC prism
PLAPO 60X0	ODPAO60
UPLAPO 10X	DP10
UPLAPO 20X	DPA20
UPLAPO 20X0	DPA20
UPLAPO 40X	DPA40
UPLAPO 40XOI	DPAO40
UPLAPO 100XOI	DP100
UPLFL 10X	DP10
UPLFL 20X	DP20
UPLFL 40X	DP40
UPLFL 100XO	DP100
UPLFL 100XOI	DP100

Appendix D-4 Setting PC Communications

Set the RS232C communication DIP switch as follows.

SW1: OFF
SW2: ON
SW3: ON
SW4: OFF
SW5: OFF
SW6: OFF
SW7: ON
SW8: ON

With the above settings, the communication rate is set to 9600 bps, data length to 7 bits, parity check to None, stop bit to 2 bits and delimiter code to “CR+LF”.
Refer to the instruction manual of your AX microscope for details.

Appendix E User Registration

FLUOVIEW manages the system setting data on a per-user basis. Use the following procedure to register yourself as a user.

TIP Log in the system with Administrator.

Register yourself as a new user.

1. From the [Start] menu, select [Programs] - [Administrative Tools(Common)] - [User Manager].
2. The [User Manager] window appears.

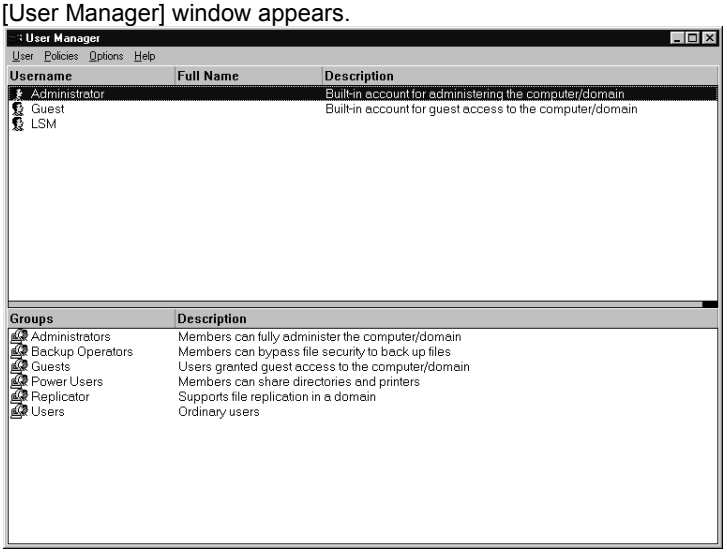


Fig. Appendix E-1 [User Manager] Window

3. Select [New User] from the [User] menu.
4. The [New User] dialog box appears.

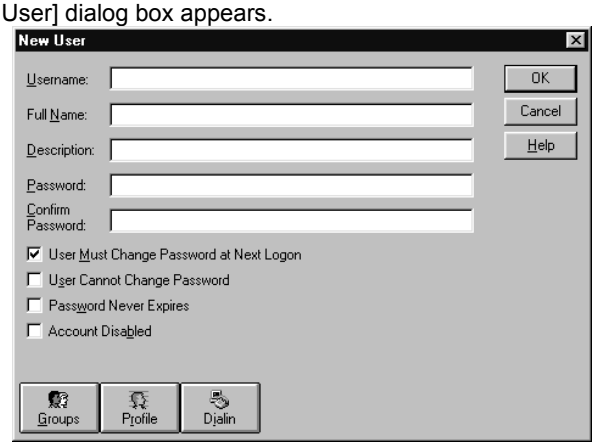


Fig. Appendix E-2 [New User] Dialog Box

5. Enter the user name in the [Username] text box.

TIP

It is not permitted to enter an existing user name or the same user name as the group name. The user name can be a character string of up to 20 characters and can contain any uppercase or lowercase characters except for the following characters.

" / \ ¥ [ ] : ; | = , + * ? < >

A user name composed only of periods (.) or space ( ) is not acceptable.

6. Enter the full name of the user in the [Full Name] text box.
7. Enter the description on the user in the [Description] text box.
8. Enter a password with up to 14 characters in the [Password] and [Confirm Password] text boxes.
9. Click the [User Must Change Password at Next Login] check box to check it.

TIP

The next time you log in with the registered user name, you can change the password.



<Group> button

10. Click the <Group> button.

    The [Group Memberships] dialog box appears.

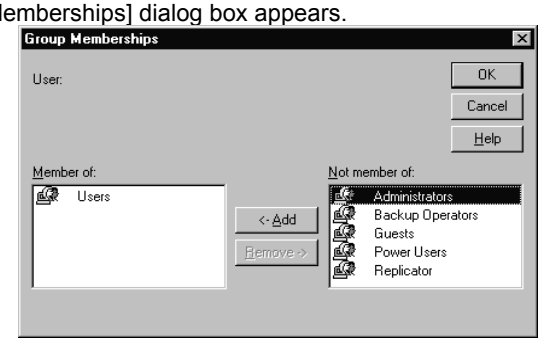


Fig. Appendix F-3 [Group Memberships] Dialog Box

11. Select [Administrators] in the [Net member of] list box.
12. Click the <Add> button.
13. Click the <OK> button.
14. Click the <OK> button.

    To register other users, repeat steps 5 to 13 for each.

Appendix F Formatting of Magnetic Optical Disk

Use the Disk Administrator tool of Windows for formatting a magnetic optical (MO) disk. Note that this tool can be used only by a person who have the authority of Administrator.

1. Click the <start> button at the bottom of the Windows screen.
2. When the [start] menu is displayed, select commands [Programs] - [Administrative Tools (Common)] - [Disk Administrator]. The window as shown below appears.

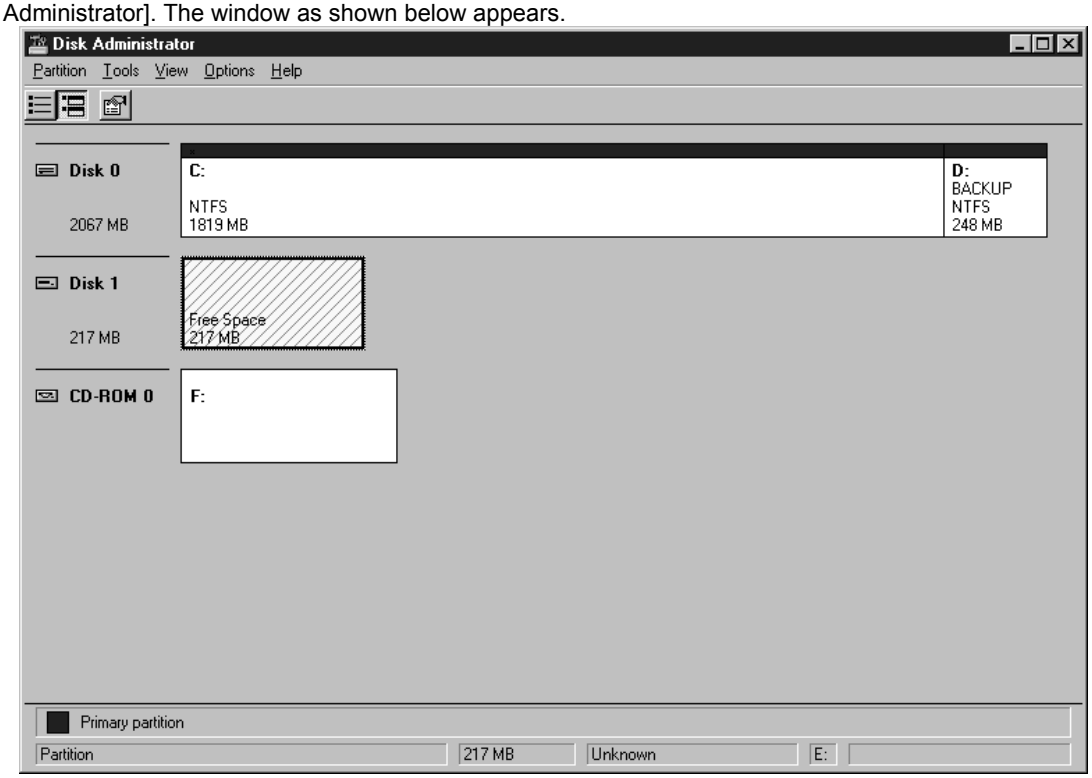


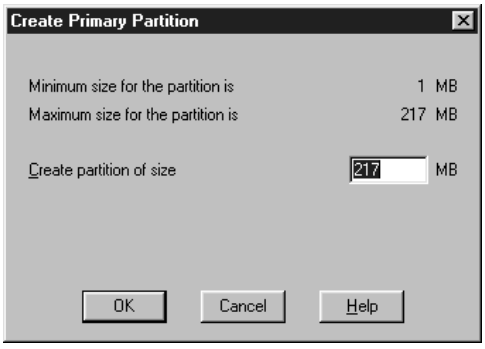
Fig. Appendix F-1 [Disk Administrator] Window

3. Select the MO disk drive in the [Disk Administrator] window.

NOTE **The drive to which the MD disk is assigned is variable depending on computers.**



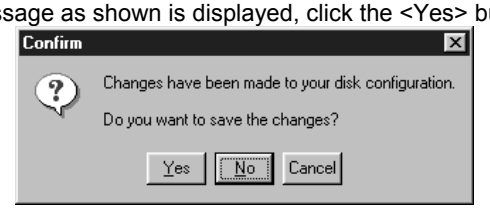
4. From the [Partition] menu of the [Disk Administrator] window, select the [Create] command. The dialog box as shown below appears.



5. Set the value of the [Create partition of size] edit box in the [Create Primary Partition] dialog box to “Maximum size”, and click the <OK> button.

6. From the [Partition] menu of the [Disk Administrator] window, select the [Commit Change know] command.

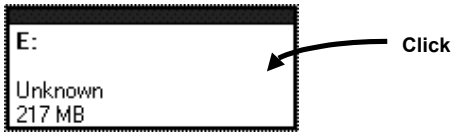
7. When the confirmation message as shown is displayed, click the <Yes> button.



8. When the following message is displayed, click the <OK> button.



9. In the [Disk Administrator] window, select the MO disk drive.



10. From the [Tool] menu of the [Disk Administrator] window, select the [Format] command. The dialog box as shown below appears.

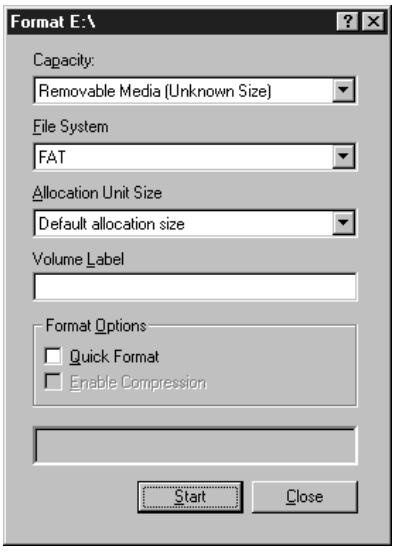


Fig. Appendix F-2 [Format] Dialog Box

11. Select [Removable Media (Unknown Size)] from the [Capacity] drop-down list in the [Format] dialog box. Then select [FAT] from the [File System] drop-down list and select [FAT] and select [Default Allocation Unit Size] from the [Allocation Unit Size] drop-down list.

12. Click the <Start> button.

13. When the confirmation message as shown below is displayed, click the <OK> button.



14. When the following message is displayed after completion of formatting, click the <OK> button.



15. Click the <Close> button in the [Format] dialog box, then select [Partition] - [Exit] in the [Disk administrator] window to exit from the operation.

Appendix G Converting Analysis Data into a Chart Using EXCEL

1. Start up Excel.
2. From the [File] menu of Excel, select the [Open] command to open the analysis data file saved after analysis using FLUOVIEW.
3. When the dialog box as shown below appears, click the [Delimited] option button in the [Original Data Type] group box, then select [Windows [ANSI]] from the [File Origin:] drop-down list.

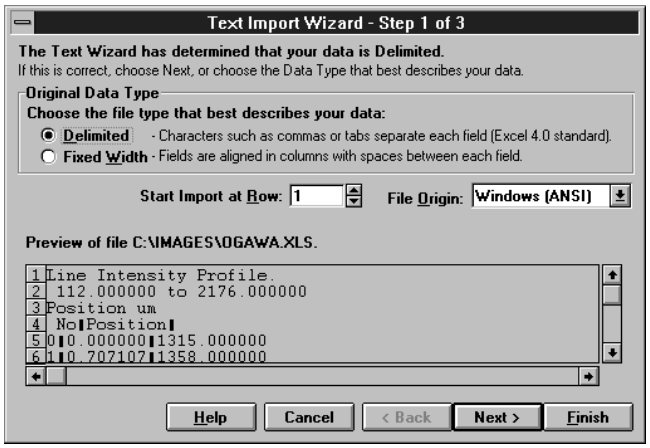


Fig. Appendix G-1 Dialog Box Displayed When File is Opened with Excel (1/3)

4. Click the <Next> button. When the dialog box as shown below appears, check the [Tab] check box in the [Delimiters] check box, then select [(none)] from the [Text Qualifier:] drop-down list.

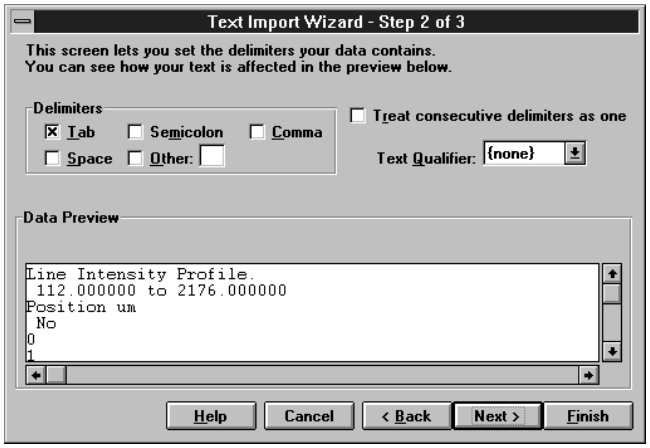


Fig. Appendix G-2 Dialog Box Displayed When File is Opened with Excel (2/3)

5. Click the <Next> button. When the dialog box as shown below appears, click the [General] option button in the [Columns Data Format] group box, then click the <Finish> button.

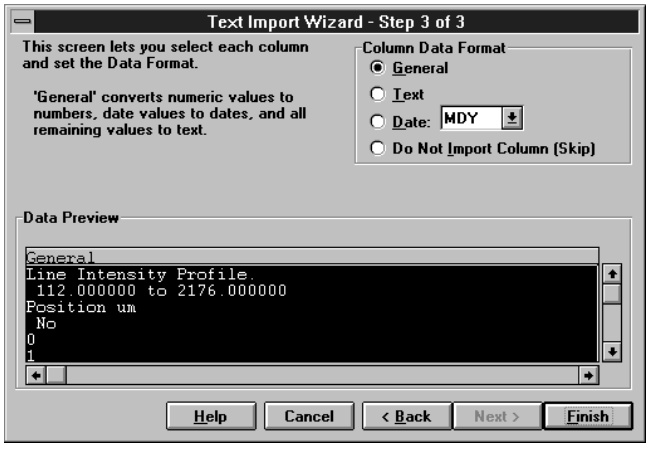
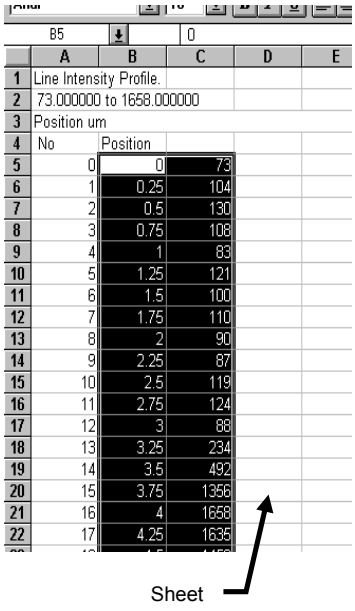


Fig. Appendix G-3 Dialog Box Displayed When File is Opened with Excel (3/3)

6. Drag the data to be converted into a chart.




<ChartWizard> button

7. Click the <ChartWizard> button.

8. On the sheet, drag the area where the chart is to be inserted.
   The dialog box as shown below appears.



Fig. Appendix G-4 Dialog Box of Chart Wizard (1/5)

9. Click the <Next> button. The dialog box as shown below appears.

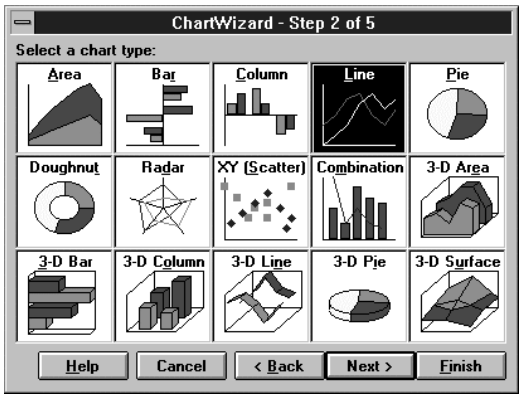


Fig. Appendix G-5 Dialog Box of Chart Wizard (2/5)

10. Select the desired chart type and click the <Next> button. The dialog box as shown below appears.

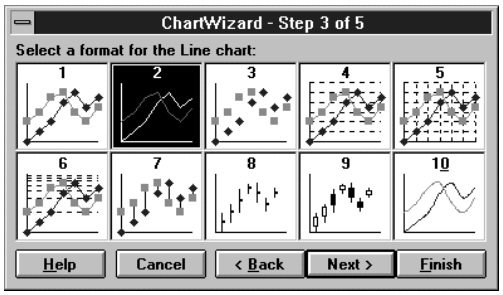


Fig. Appendix G-6 Dialog Box of Chart Wizard (3/5)

11. Select the desired chart format and click the <Next> button. The dialog box as shown below appears.

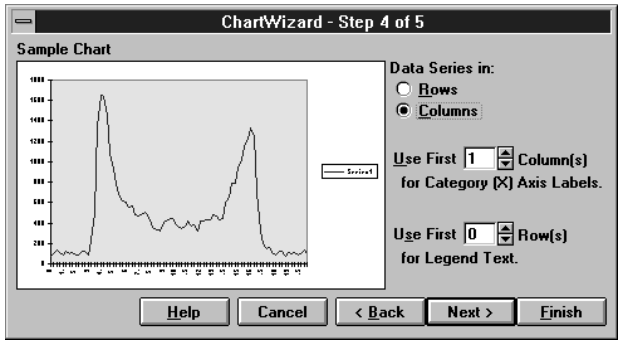


Fig. Appendix G-7 Dialog Box of Chart Wizard (4/5)

12. Click the [Columns] option button under [Data Series in:].

13. Set the data column number that you want to use as the X-axis label position in the [Use First Column(s)] text box.

14. Click the <Next> button. The dialog box as shown below appears.

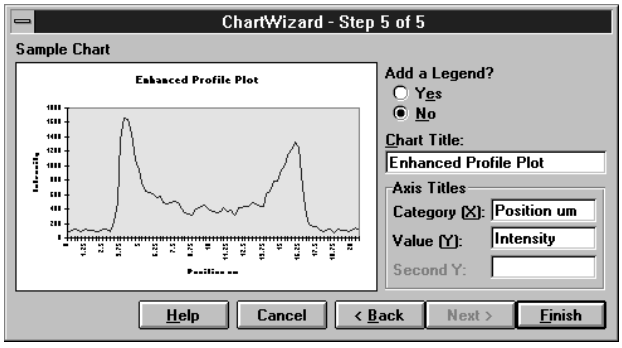


Fig. Appendix G-8 Dialog Box of Chart Wizard (5/5)

15. Enter the chart title in the [Chart Title] text box.

16. Enter the X-axis label in the [Category (X):] text box and the Y-axis label in the [Value (Y):] text box.

17. Click the <Finish> button. A chart will be drawn on the sheet.

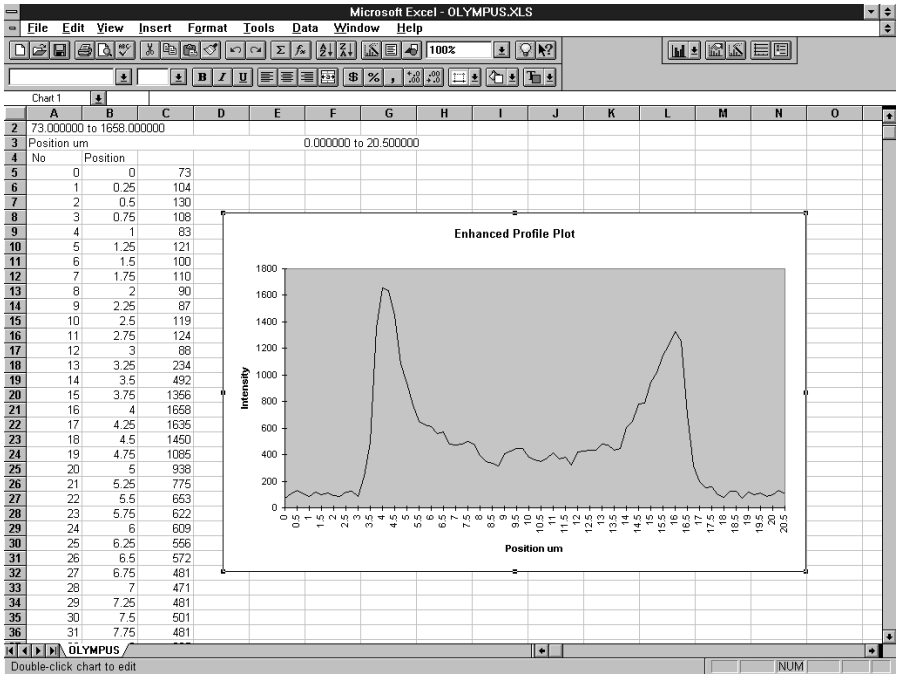


Fig. Appendix G-9 Sheet with Inserted Chart

TIP

When the mouse is clicked on the chart, black handles will appear around the chart.

The following operations are available in this condition.

- Moving the chart

  Place the mouse pointer on the chart and drag it.

- Resizing the chart

  Place the mouse pointer on one of the handles around the chart and drag it.

To return to the original condition, click a place outside the chart.

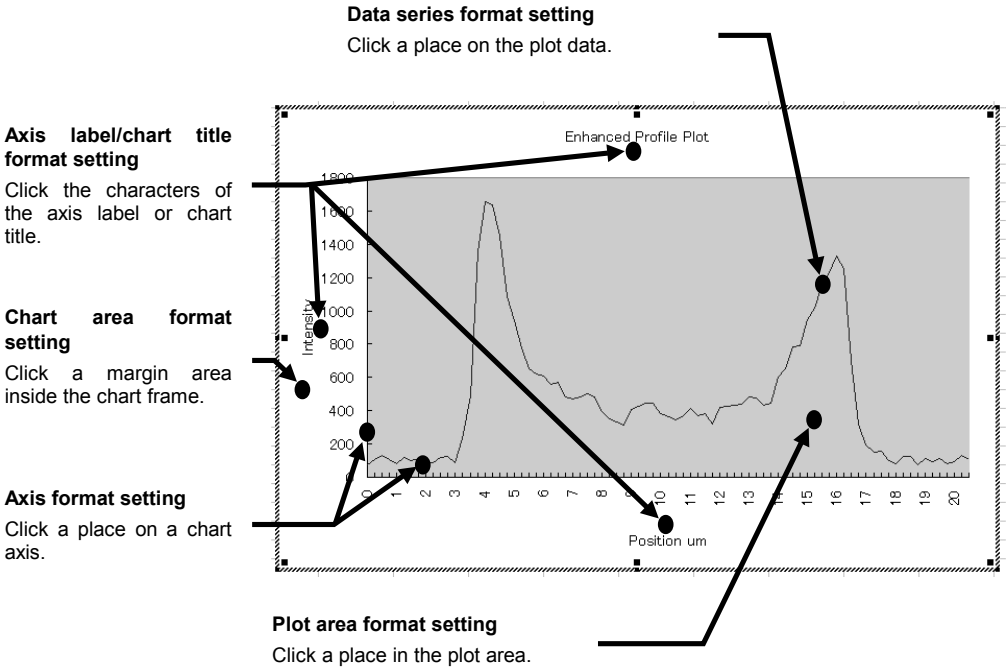
To enter reference text, use the “reference text” style.

TIP

When the mouse is double-clicked on the chart, a frame composed of oblique lines appear around the chart. (The frame may become a window when the chart is large.)

The format settings are available in this condition.

1. Click one of the following positions to display black handles.



2. Double-click. The dialog box for setting the selected format appears. Set the desired format and click the <OK> button.

   (Refer to the Excel manuals for details.)

To return to the original condition, click a place outside the chart.

Appendix H USER REGISTRATION OF FV500

FLUOVIEW FV500 can store the system setup information (PMT Voltage, Gain, Offset, etc.) on a per-user basis.

To make this possible, you have to register yourself as a user and log in personally when starting up the FV500 software.

Appendix H-1 User Registration

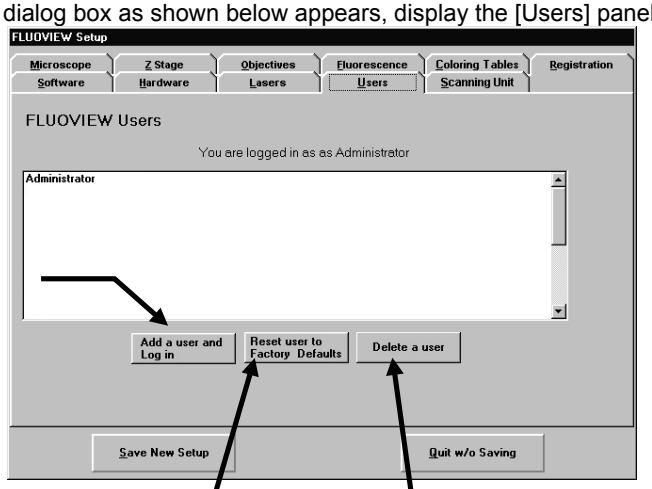
Register yourself as a new user.



[FLUOVIEW Setup] icon

1. Double-click the [FLUOVIEW Setup] icon on the desktop.

   When a dialog box as shown below appears, display the [Users] panel at the front.

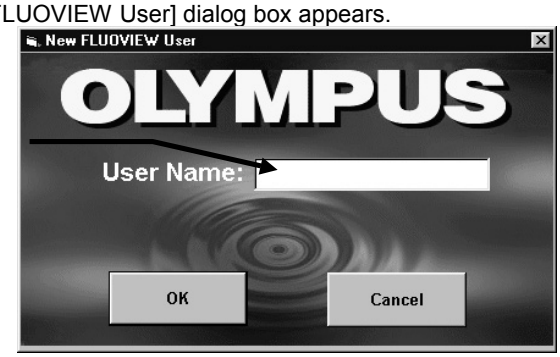


<Add a user and Log in> button
Used to add a new user.

<Reset user to Factory Defaults> button
Resets the system setups of the users selected in the list box to the factory defaults.

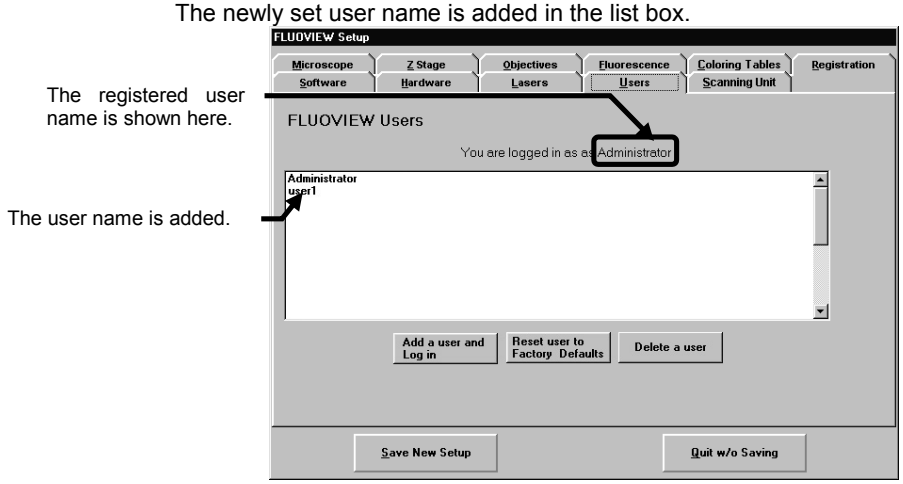
<Delete a user> button
Deletes the user selected in the list box.
(The Administrator and the user who log in now cannot be deleted.)

2. Click the <Add a user and Log in> button.
3. The [New FLUOVIEW User] dialog box appears.



[User Name:] text box
Enter the user name to be registered.

4. Enter the user name in the [User Name] text box and click the <OK> button.
   The newly set user name is added in the list box.



5. To register other users, repeat steps 3 to 5 for each one.
6. Click the <Save New Setup> button or <Quit w/o Saving> button to close the dialog box.

TIP

The Administrator is the user name which saves the factory defaults of the system setup.

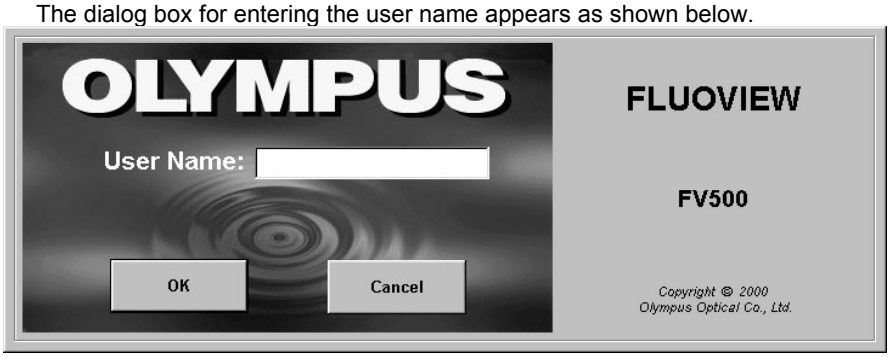
Appendix H-2 Logging into the FV500

After the user name has been registered and the FV500 started, a dialog box for entering the user name appears.
Enter the user name to log in the FV500.



[FLUOVIEW] icon

1. Double-click the [FLUOVIEW] icon on the desktop.
   The dialog box for entering the user name appears as shown below.



2. Enter the user name in the [User Name:] text box and click the <OK> button.
3. The FLUOVIEW software starts.

TIP

If no user name has been registered, the [FLUOVIEW User Login] dialog box is not displayed, but the system is automatically started for the Administrator.

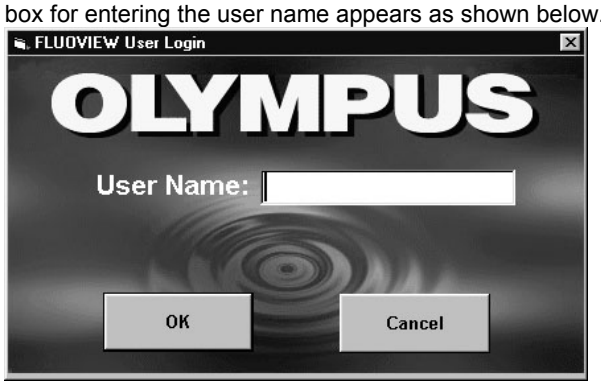
Appendix H-3 Deleting a User



[FLUOVIEW Setup] icon

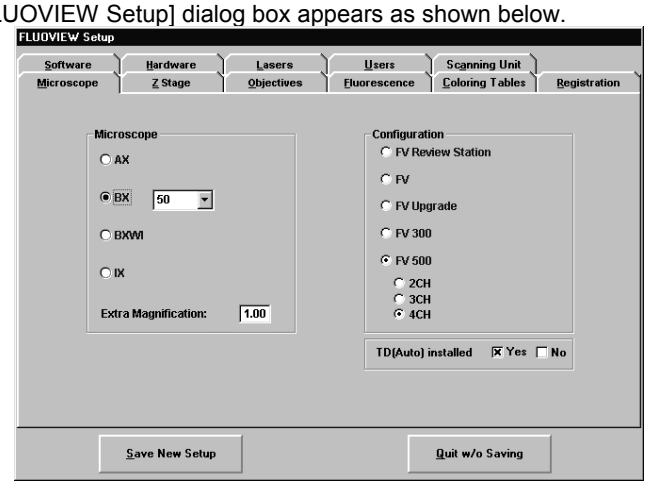
1. Double-click the [FLUOVIEW] icon on the desktop.

   The dialog box for entering the user name appears as shown below.

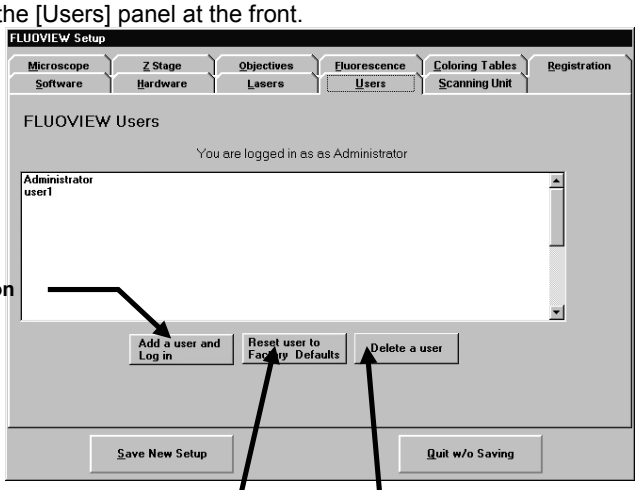


2. Enter “Administrator” in the [User Name:] text box and click the <OK> button.

   The [FLUOVIEW Setup] dialog box appears as shown below.



3. Display the [Users] panel at the front.



<Add a user and Log in> button

Used to add a new user.

<Reset user to Factory Defaults> button

Resets the system setups of the users selected in the list box to the factory defaults.

<Delete a user> button

Deletes the user selected in the list box.

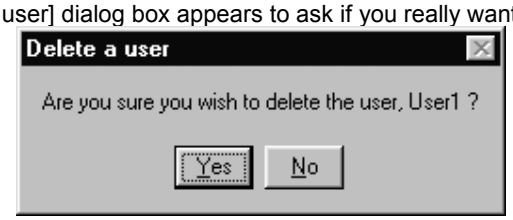
(The Administrator and the user who log in now cannot be deleted.

4. Select the user name to be deleted from the list box.

5. Click the <Delete a user> button.

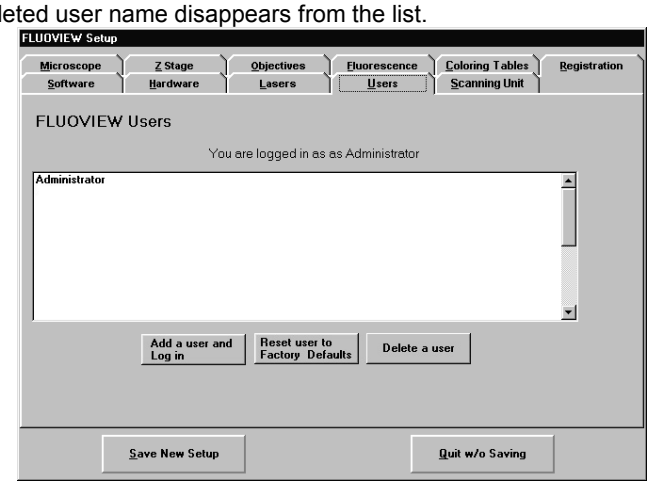
NOTE **The Administrator cannot be deleted from the list.**

6. The [Delete a user] dialog box appears to ask if you really want to delete the user.



7. Click the <Yes> button if you want to delete the user or the <No> button if you do not.

   The deleted user name disappears from the list.



NOTE **The user is deleted at the moment the <OK> button is clicked.**

8. Click the <Save New Setup> button or <Quit w/o Saving> button to close the dialog box.

Appendix I Change of Default Folder for [File I/O] Panel

FLUOVIEW FV500 usually opens the default folder for the [File I/O] panel (C:\ FLUOVIEW \ IMAGES) to save acquired images or load saved images.

The default folder for saving an image can be changed or a desired folder can be specified directly when loading a saved image.

TIP

Change the default folder only when required.

There is no need of change if folder C:\ FLUOVIEW \IMAGES is all right.

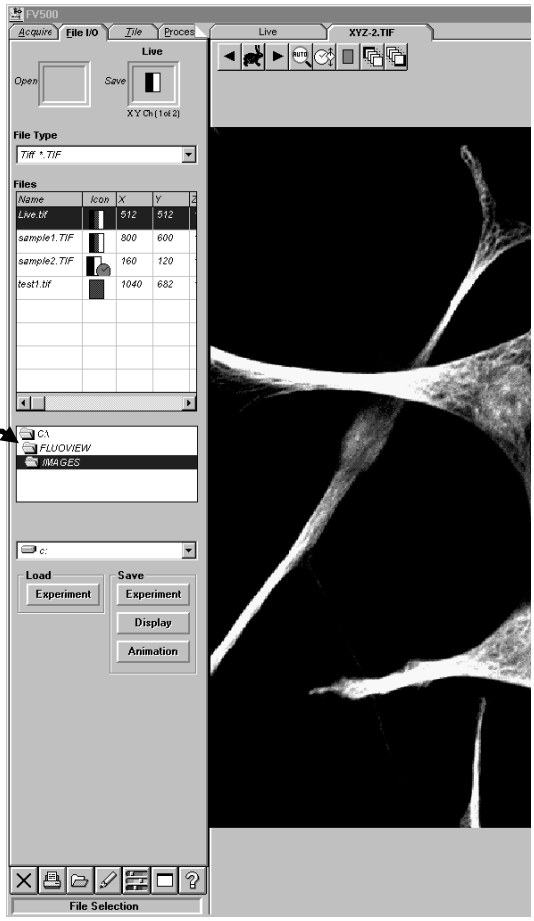
TIP

Folder names are delimited with " \".

For example, "C:\ FLUOVIEW \IMAGES" means folder "IMAGE" in folder "FLUOVIEW" in "drive C".

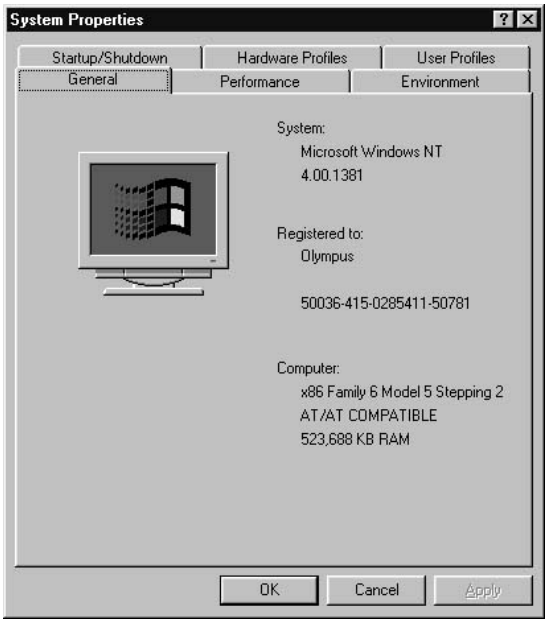
- When the default folder is C:\ FLUOVIEW \IMAGES

Default folder "C:\ FLUOVIEW \ IMAGES" is being open.



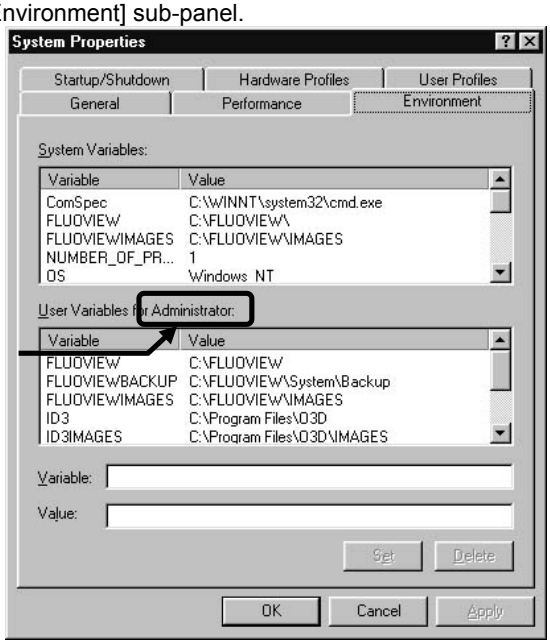
A different default folder can be set for each of the users logging in Windows NT.

1. Click the [Start] button to open the [Start] menu. Then select commands [Settings] - [Control Panel] - [System]. The [System Properties] dialog box appears as shown below.

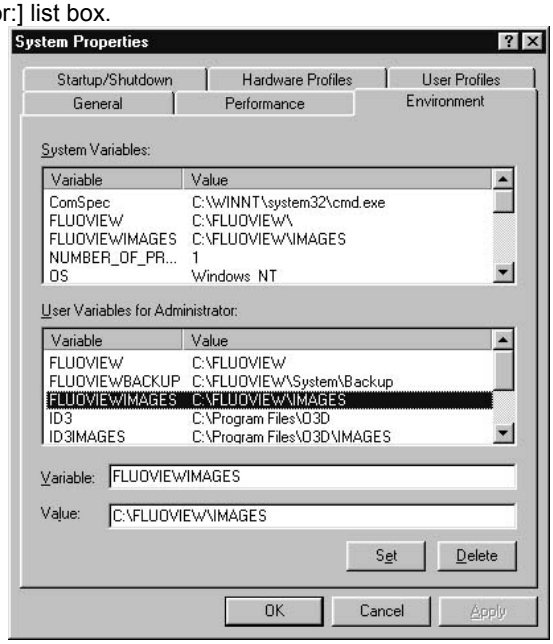


2. Select the [Environment] sub-panel.

User name logging in Windows NT.



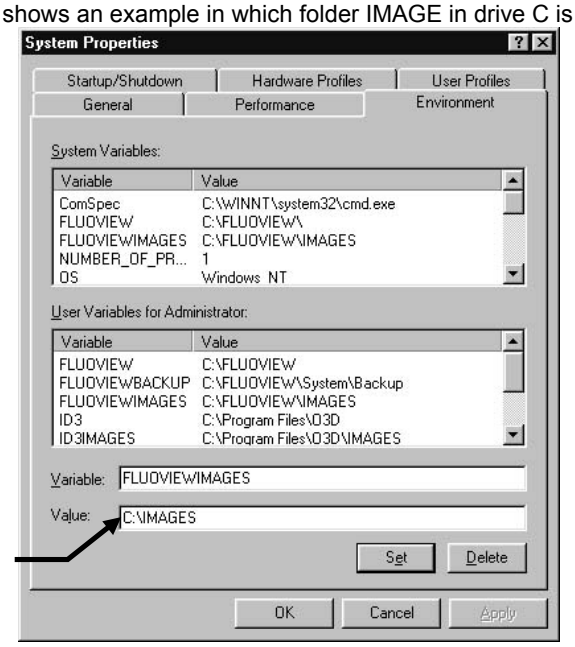
3. Select [FLUOVIEWIMAGES] under [Variable] in the [User Variables for Administrator:] list box.



TIP

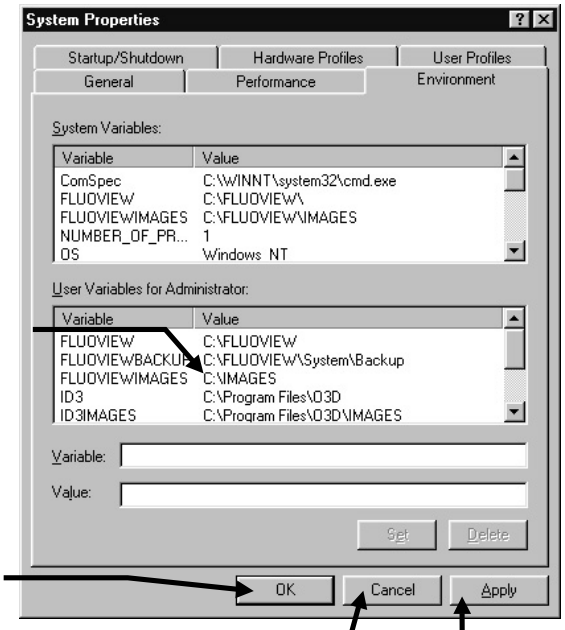
"Administrator" of [User Variables for Administrator:] is variable depending on the user name logging in Windows NT.

4. In the [Value:] text box, enter the path name of the default folder to be changed by delimiting the drive and folder names using backslashes (\ ).
(The figure shows an example in which folder IMAGE in drive C is entered.)



[Value:] text box
Enter the path name of the default folder.

5. Click the <Set> button



Change is reflected.

<OK> button
Saves the change and closes the dialog box.

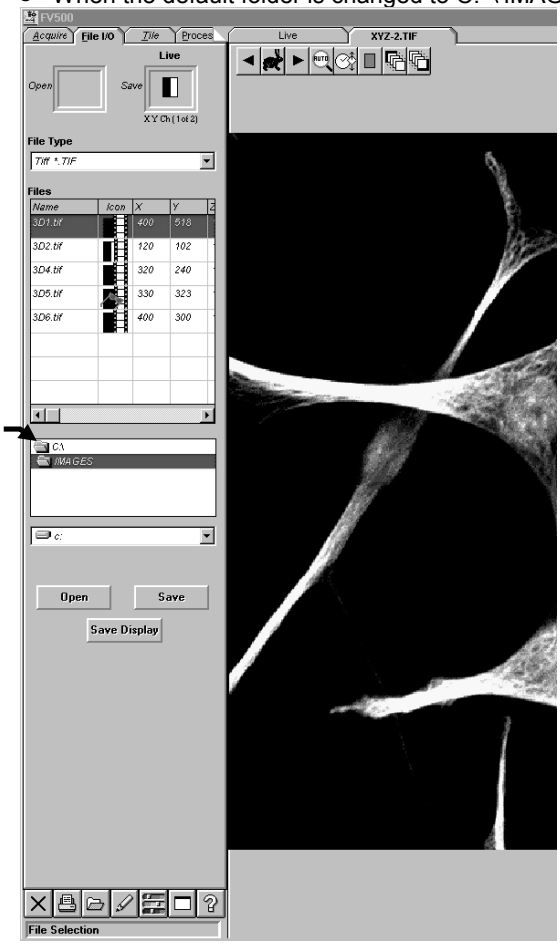
<Cancel> button
Closes the dialog box without saving the change.

<Apply> button
Saves the change without closing the dialog box.

6. Click the <OK> button to close the [System Properties] dialog box.
   Now, the default folder for the [File I/O] panel will be changed from the next time you start FLUOVIEW.

- When the default folder is changed to C: \ IMAGES

New default folder C \IMAGES is being open.



TIP To reset the default folder to the factory setup, enter "C: \ FLUOVIEW \ IMAGES" in the [Value] text box.

NOTE **Do not change the setup after "FLUOVIEWIMAGES" in the [User Variables for <username>] list box. If erroneous setting is made here, the system will be unable to be started up.**

Appendix J List of Functions in the [Active Overlays] Dialog Box

Active Overlays is a kind of overlay function displayed on an image.
Active Overlays does not simply show the entered characters, but searches the image data related to the keyword specified in < > and displays the data values on the image.

The following setups are required to enable the use of Active Overlays.

Appendix J-1 Coordinate Position Data

Appendix J-1-1 X-Coordinate

The X-coordinate position with respect to the top left corner of the screen is displayed.

«Syntax»

<x[[hotspot] [raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

«Setup Procedure»

1. Enter “x” as the first character inside < >.
2. Add the following characters to set the display method.

   hotspot : Display measurement points with “+” markings.
   raw : Display data in pixel values.
   calibrated : Display data in numerical values (µm).
   units : Display the unit (pixels/µm).

«Examples»

<Input character strings>	<Displayed strings (** represent figures.)>
<x raw value>	**
<x hotspot raw value>	+**
<x hotspot calibrated value units>	+**µm

TIP

The detailed display procedure is described in section 2-11-3, “Viewing the X- or Y-Coordinate Position of Image” in Volume [OPERATION].

Appendix J-1-2 Y-Coordinate

The Y-coordinate position with respect to the top left corner of the screen is displayed.

«Syntax»

<y[[hotspot] [raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

«Setup Procedure»

1. Enter “y” as the first character inside < >.
2. Add the following characters to set the display method.

hotspot	: Display measurement points with “+” markings.
raw	: Display data in pixel values.
calibrated	: Display data in numerical values (μm).
units	: Display the unit (pixels/μm).

«Examples»

<Input character strings>	<Displayed strings (** represent figures.)>
<y raw value>	**
<y hotspot raw value>	+**
<y hotspot calibrated value units>	+**μm
<x hotspot raw value>,<y raw value>	+(**,**)

TIP

The detailed display procedure is described in section 2-11-3, “Viewing the X- or Y-Coordinate Position of Image” in Volume [OPERATION].

Appendix J-1-3 Other

Positions can also be displayed by entering the following keywords in the syntax in place of the X- and Y-coordinate positions described in sections J-1-1 and J-1-2.

1 Z Position

With cross-section related images such as XYZ images, the Z position with respect to the first image can be displayed.

«Syntax»

<z[[raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

2 T Position

With time-related images such as XYZ images, the T position with respect to the first image can be displayed.

«Syntax»

<t[[raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

3 Animation

With animation images created in the [Visualize] panel, the position with respect to the first image can be displayed.

«Syntax»

<Animation[[raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

Appendix J-2 Intensity Data

The intensity value can be displayed.

When images are overlapped, the intensity value of each image is accompanied with the channel number placed after it.

«Syntax»

<intensity[[hotspot] [raw/calibrated] value [units]]>

Arguments inside [ ] can be omitted.

If [raw/calibrated] is omitted, the same setting as when [calibrated] is specified will be applied.

«Setup Procedure»

1. Enter “intensity” as the first character inside < >.
2. Add the following characters to set the display method.

   hotspot : Display measurement points with “+” markings.

   raw : Display data in values between 0 and 4095.

   calibrated : Display data in numerical values (μm).

   units : Display the unit if this has been set.

   (Same as the concentration computation in FV-TIEMPO)

«Examples»

<Input character strings>	<Displayed strings (** represent figures.)>
<intensity raw value>	·····**
<intensity hotspot raw value>	·····***(1),**(2) (When images are overlapped)

TIP The detailed display procedure is described in section 2-11-2, “Viewing the Intensity Value of Image” in Volume [OPERATION].

Appendix J-3 Other

The following image data can be displayed in addition to the X- and Y-coordinate positions and intensity value.

Appendix J-3-1 Channel Number

The channel number can be displayed.

When images are overlapped, the displayed channel numbers are connected by the "+" markings.

«Syntax»

<Channel>

Appendix J-3-2 Objective Power

The objective power used in image capturing can be displayed.

«Syntax»

<Objective>

Appendix J-3-3 Date of Image Capturing

The date at which the image was captured can be displayed.

«Syntax»

<Date>

Appendix J-3-4 Time of Image Capturing

The time of the day at which image was captured can be displayed.

«Syntax»

<Time>

Appendix J-3-5 Image File Name

The image file name can be displayed.

«Syntax»

<Name>

MAINTENANCE

On This Volume

This volume describes the user maintenance procedures of the FLUOVIEW FV500 system.
Please read this volume so that you can use the system for an extended period of time.

1 Software Setup 1-1

1-1 Re-setup or Updating of the Software.....1-1
1-2 New Setup of the Software.....1-3
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1-3-1 Overall Setting of FLUOVIEW 1-6
1-3-2 Setting the [BX Control Panel]..... 1-14
1-4 Adding the dyeing method 1-20

2 Maintenance of Major System Units 2-1

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2-2 UV-Ar Laser.....2-2
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1 Software Setup

The FLUOVIEW software has been set up before it is delivered to the user.

A CD-ROM containing the software program is provided with FLUOVIEW. This CD-ROM is intended for use when re-setup is required due to a fault or when the user wants to set up the software newly.

When the FLUOVIEW software is updated, the user is also requested to update the software in use accordingly.

For re-setup or updating of the software, see section 1-1, “Re-Setup or Updating of the Software” below and follow the procedures given in it.

When you want to set up the software newly, see section 1-2, “New Setup of the Software” below and follow the procedures given in it.

TIP

To set up the FLUOVIEW software, it is necessary that the computer in use already has Microsoft Windows NT Version 4.0 (English version) installed in its hard disk.

A vacant space of 100 MB is required for the setup.

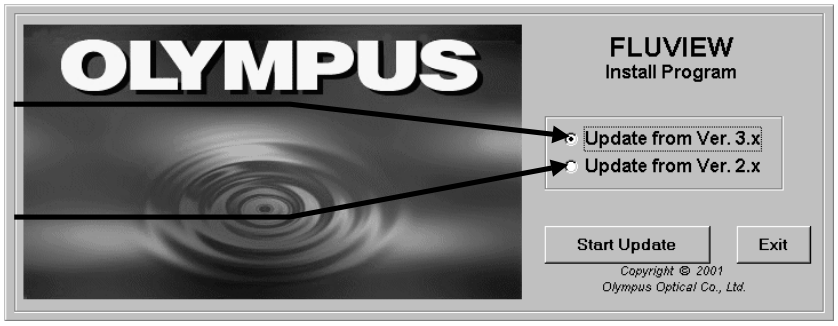
1-1 Re-setup or Updating of the Software

Save the current settings and remove the current software before starting re-setup or updating with the following procedures.

1. Insert the FLUOVIEW setup CD-ROM in the CD-ROM drive.
   Then the dialog box as shown below appears.

[Update from Ver.3.x] option button
When updating from FV software after ver3.0, select this option button.

[Update from Ver.2.x] option button
When updating from FV software of ver2.0 to ver2.9, select this option button.



2. Click the option button of the version of FV software installed, and click the <Start Update> button.

3. [Update FV Software] dialog box is displayed and starts the current settings.
   For save, carry out according to a wizard.

NOTE

Current settings are performed after <Start Update> button selection. Be sure to save the current settings before proceeding to the removal of the software.

If the software is removed without saving the current settings, the settings in the software program obtained by re-setup will be reset to the initial settings.

4. Continuously, deletion of software is started.
   For deletion, carry out according to a wizard.

5. Continuously, installation of the software of a new version is started.
   Refer to 1-2 “New Setup of the Software” for details.

1-2 New Setup of the Software

The following procedure allows you to set up the FLUOVIEW software in a computer you use.

1. Load the FLUOVIEW setup CD-ROM in the CD-ROM drive.

2. Select the [Start] button to display the [Start] menu, then select commands [Settings] - [Control Panel] - [Add/Remove Programs]. The [Add/Remove Programs Properties] dialog box as shown below will appear.

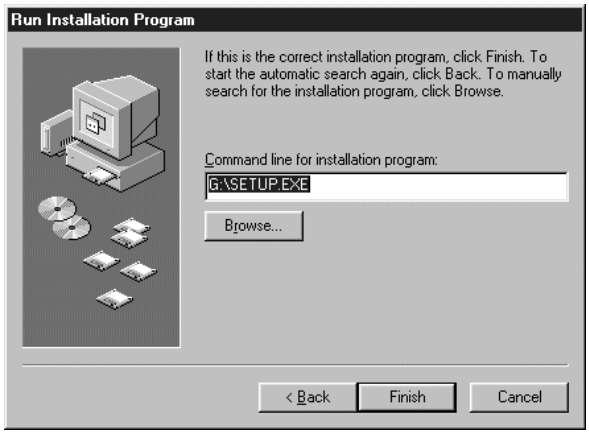


3. Select the <Install> button. The dialog box as shown below will appear.



4. When the [Install Program From Floppy Disk or CD-ROM] dialog box appears, select the <Next> button.

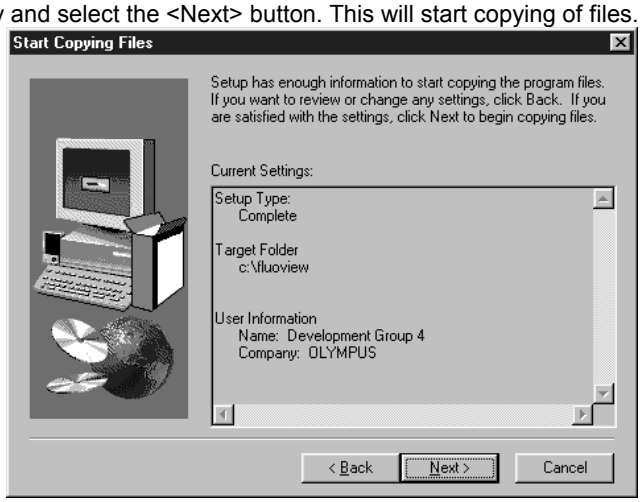
5. When the [Run Installation Program] dialog box appears, ensure that the "<CD-ROM drive name>\SETUP.EXE" is displayed correctly and select the <Finish> button.



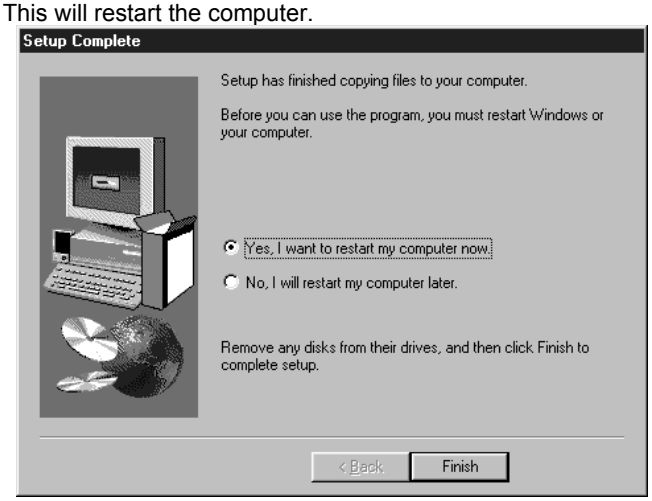
6. When the [Choose Destination Location] dialog box appears, confirm the setup destination drive name and directory and select the <Next> button.



7. The [Start Copying Files] dialog box appears. Confirm the installation destination directory and select the <Next> button. This will start copying of files.



8. When the setup has completed, the [Setup Complete] dialog box appears. Select the [Yes, I want to restart my computer now.] option button and press the <Finish> button. This will restart the computer.



1-3 Setting the System Configuration

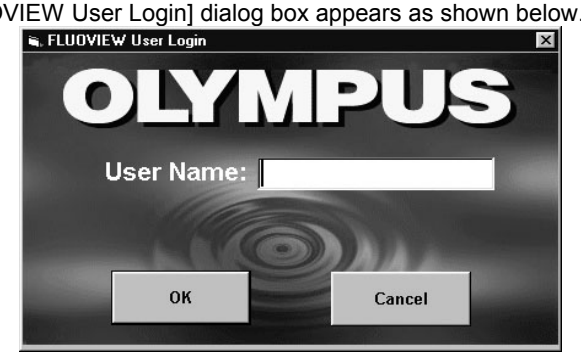
If it is required to change the settings after having set up the software, perform the following procedure.

1-3-1 Overall Setting of FLUOVIEW



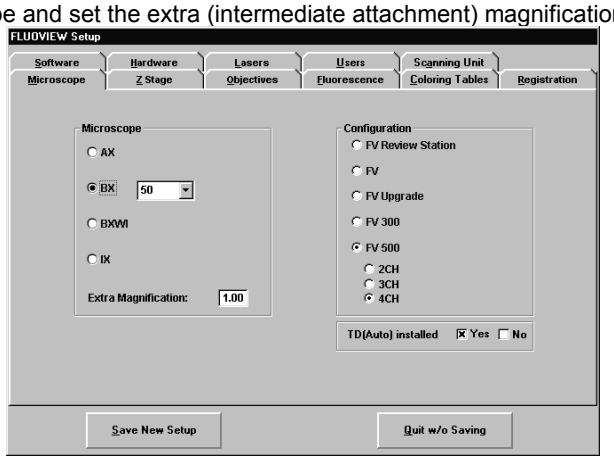
[FLUOVIEW Setup] icon

1. Double-click the [FLUOVIEW Setup] on the desktop.
   The [FLUOVIEW User Login] dialog box appears as shown below.



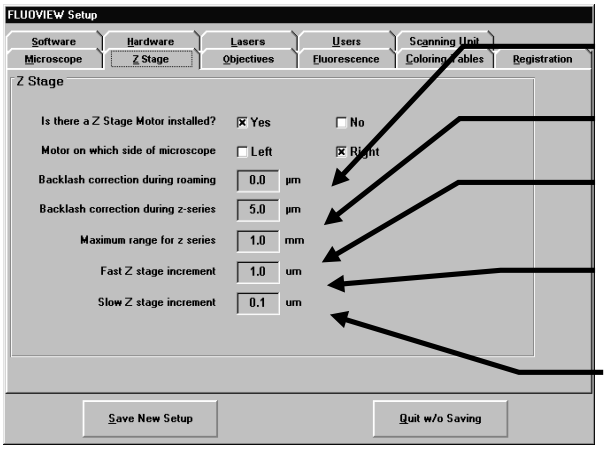
2. Enter the user name in the [User Name :] text box and click the <OK> button to log into FLUOVIEW FV500.
   Refer to Appendix H-2 “Logging into the FV500” for details.

3. The dialog as shown below appears for use in saving the system configuration.
   First, display the [Microscope] panel in the front position, select the type of microscope and set the extra (intermediate attachment) magnification to 1.0.



Selecting “BX51, 52” or “BX51,52WI” and “Automatic” in the [Microscope] group box or “BX61, 62” or “BX61,62WI” in the [Microscope] group box shows the [Microscope Setup] dialog box to edit the BX operation panel (BX Control Panel). For details, see section 1-3-1 “Setting the [BX Control Panel]”.

4. Display the [Z Stage] panel in the front position, and check and set the presence of motor controller, positioning of the motor controller, backlash and moving amount of the Z motor.



Set the backlash of motor operation in the [Z Stage] sub-panel.

Set the backlash during scanning involving the Z stage.

Set the range where the stage can move during the Z motor operation

Set the moving amount assigned to the <Z stage coarse adjustment> buttons.

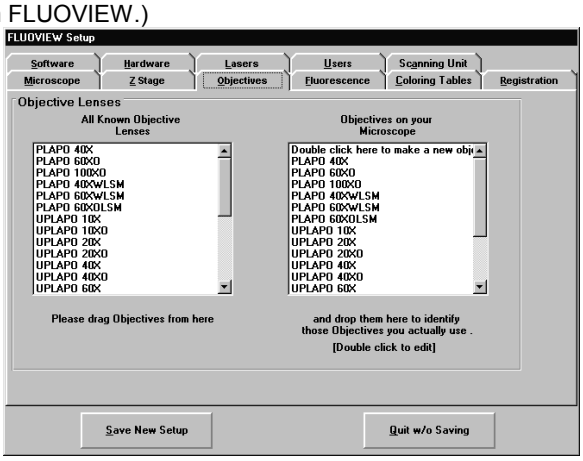
Set the moving amount assigned to the <Z stage fine adjustment> buttons.

TIP

The [Maximum range for Z series (mm)] text box accepts a setting value between 1.0 and 3.0 (mm).
The values in the [Start Z] and [Stop Z] text boxes in the [Z Stage] sub-panel are set according to the value set there.
For example:
When “1.0” (mm) is set as the maximum range for Z series, the minimum value for Z starting becomes -500 (µm) and the maximum value for Z stopping becomes 500 (µm).

5. Display the [Objectives] panel in the front position.

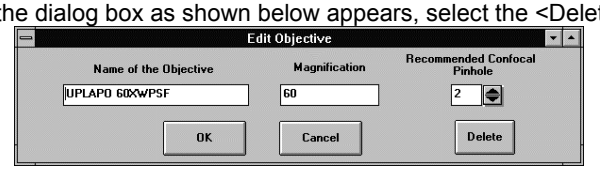
In this panel, select the items to be included in the list of objectives used by the FLUOVIEW application. (Each user should set the objectives that the user wants to use with FLUOVIEW.)



<To delete an unnecessary objective from the list>

In the list on the right, double-click the objective to be deleted from the list.

When the dialog box as shown below appears, select the <Delete> button.



<To change the details (objective name, pinhole No., magnification) of an objective>

Double-click the objective for which you want to change the detailed information. When the dialog box appears, change the desired information items and select the <OK> button.

<To add an objective to the list>

Double-click “Double Click here...” at the top of the list on the right. When the dialog box appears, set the detailed information on the new objective and select the <OK> button.

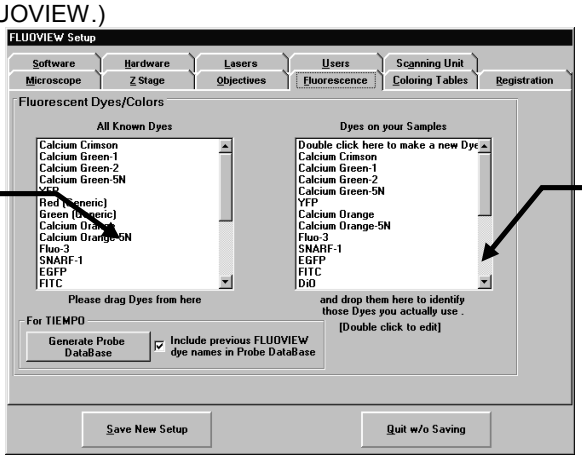
<To add one of the objectives in the list on the left to the list on the right>

Place the mouse pointer on the objective to be added in the list on the left, and drag it to the list on the right.

6. Display the [Fluorescence] panel in the front position.

   In this panel, select the items to be included in the list of dyes used by the FLUOVIEW application. (Each user should set the dyes that the user wants to use with FLUOVIEW.)

List of all dyes available at present

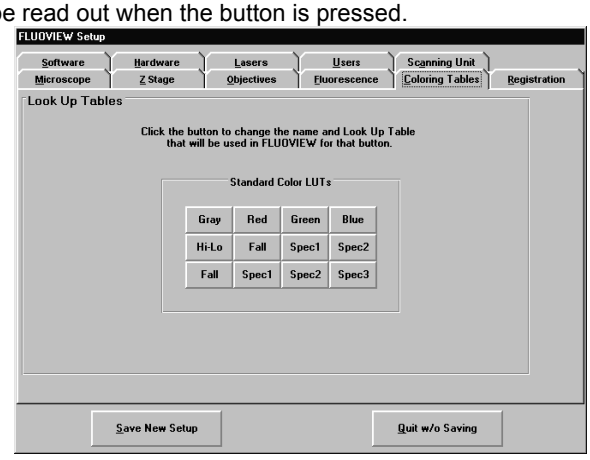


List of dyes that can be used during FLUOVIEW operation

   The setting procedure is similar to the objective setting procedure ([Objectives] panel).

7. Display the [Coloring Tables] panel in the front position.

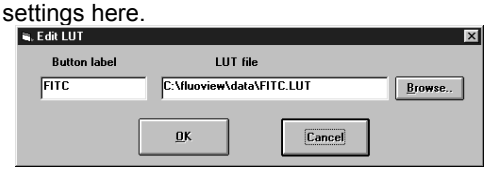
   In this panel, select the characters to be displayed in each of the 8 buttons displayed in the [Color Tool] dialog box of the FLUOVIEW application and the LUT file to be read out when the button is pressed.



<To change the button settings>

Press the button to indicate that you want to change the setting. When the dialog box as shown below appears, specify the button name and LUT file name and select the <OK> button.

The LUT files which have been used up to Ver 1.0* (FITC, etc.) are supplied with this version in the same names as before. If you want to use such files, change their settings here.



8. Display the [Software] panel in the front position.

In this panel, select the initial magnification status when images are to be displayed in [Display] panels. This panel is also used to set the mode of comment overlay in image and to set the numbers of [Function] and [Display] panels that can be displayed together.

[Default Image Zoom in Single View] group box

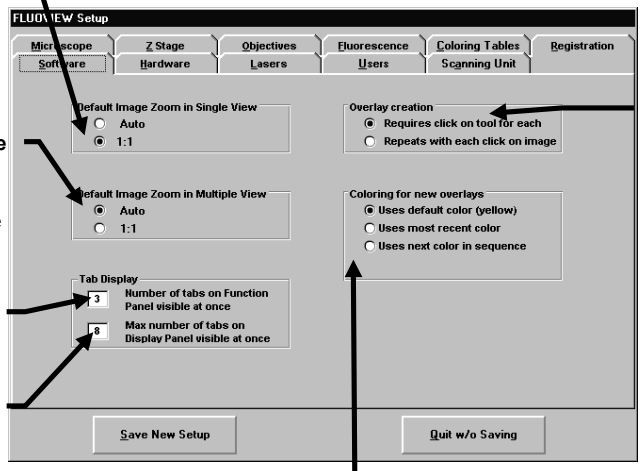
Select the initial status of display magnification when a single image is to be displayed in a single [Display] panel.

[Default Image Zoom in Multiple View] group box

Select the initial status of display magnification when multiple images are to be displayed by displaying multiple [Display] panels together.

Set the number of [Function] panels that can be displayed at a time.

Set the number of [Display] panels that can be displayed at a time.



[Overlay creation] group box

When comment is to be overlaid in image, select either the mode allowing repeated entries of comment per Annotation tool selection or the mode allowing single entry of comment.

[Coloring for new overlays] group box

Set the color of the comment to be overlaid in image in the [Display] panel.

9. Display the [Hardware] panel in the front position.

   In this panel, select Enabled/Disabled to enable/disable the automatic black-level adjustment, enter the values for delay timing for TD unit, and enter the maximum duration for continuous bi-directional scanning when setting fast scan mode.

[Delay Timing for TD Unit] group box

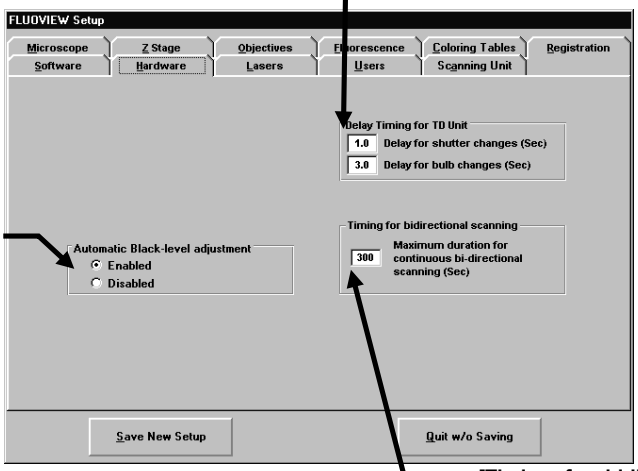
This data should usually be left in the default values and does not have to be changed.

[Delay shutter changes (sec)] text box

Enter the opening/closing wait time of the shutter in the TD unit in seconds.

[Delay for bulb changes (sec)] text box

Enter the ON/OFF wait time of the lamp in the TD unit in seconds.



[Automatic Black-level adjustment] group box

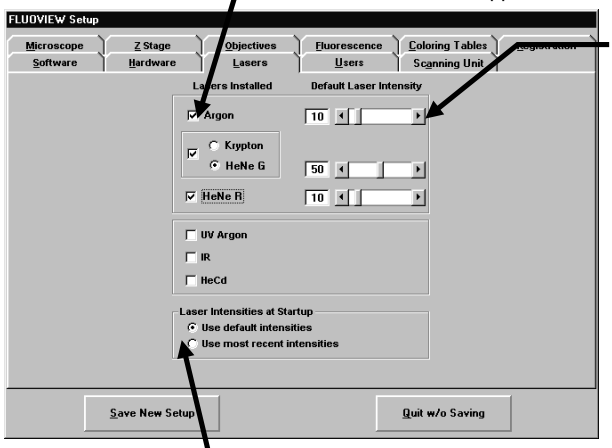
Select Enabled/Disabled to enable/disable the automatic black-level adjustment.

[Timing for bidirectional scanning] group box

Enter the maximum duration for continuous bi-directional scanning (in seconds) when setting fast scan mode.

10. Display the [Lasers] panel in the front of position.

    Set the lasers to be used with the FLUOVIEW application and the initial ND filter values here.



[Laser Install] check box

Select and check the lasers to be used with the FLUOVIEW application.

[Default Laser Intensity] scale

Set the initial ND filter value at the start of the FLUOVIEW application.

[Laser Intensities at Startup] group box

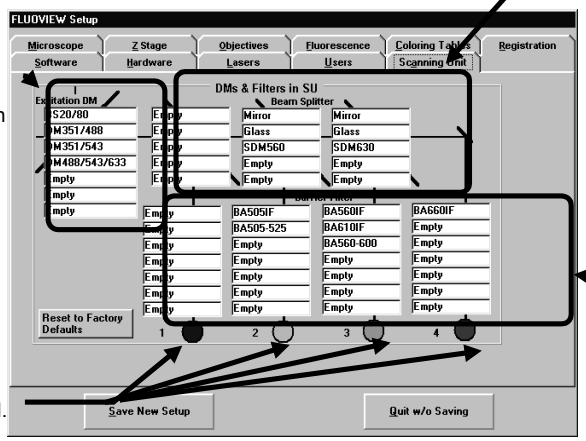
Set the laser intensities when starting up the FLUOVIEW application.

[User default intensities] option button
Select to use the default intensity set in the [Default Laser Intensity] scale above.

[Use most recent intensities] Option button
Set to use the intensity that was used in the last observation.

11. Display the [Scanning Unit] panel in the front of position.

    Set the filters to be used with the Scanning unit.



[Excitation DM]

Sets the excitation filter of each channel.

And corresponds to the order of the scanning unit from the top to bottom.

[Beam Splitter]

Sets the beam splitter of each channel.

From the left, this shows the beam splinter of Ch1, Ch2, and Ch3.

And corresponds to the order of the scanning unit from the top to bottom.

[Barrier Filter]

Sets the barrier filter of each channel.

And corresponds to the order of the scanning unit from the top to bottom.

Shows each channel.

12. The [Registration] panel have been set at the factory and do not need to be changed here.

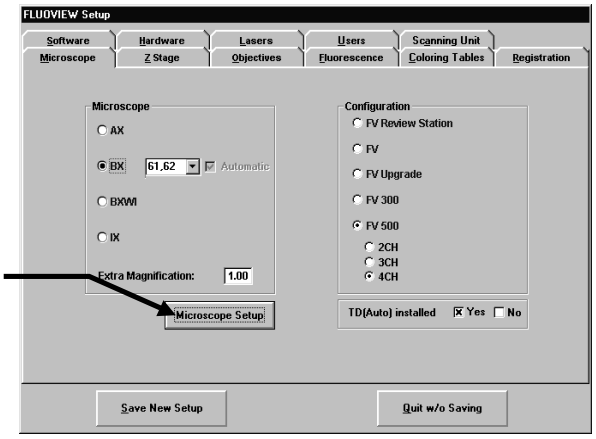
13. After completing the setup, select the <Save New Setup> button on the bottom left of the panel.
    (Selecting <Quit w/o Saving> exits the panel without saving the system setup.)

1-3-2 Setting the [BX Control Panel]

The <Microscope Setting> button as shown below appears when selecting “BX 51,52” or “BX51,52WI” and checking the [Automatic] check box or selecting “BX61,62” or “BX61,62WI” in the [Microscope] group box in the [Microscope] panel in the [FLUOVIEW Setup] dialog box.

<Microscope Setup> button

Displays the [BX Control Panel] window.



Clicking the <Microscope Setup> button displays the [Microscope Setup] window as shown below.

[Filter Turret] group box

Sets up the name and color of the filter turret for reflected light observation.

[Filter Turret] group box

Sets up the name and color of the filter turret for visual observation.

[Mirror Unit] group box

Sets up the name and color of the cube turret.

[Nosepiece] group box

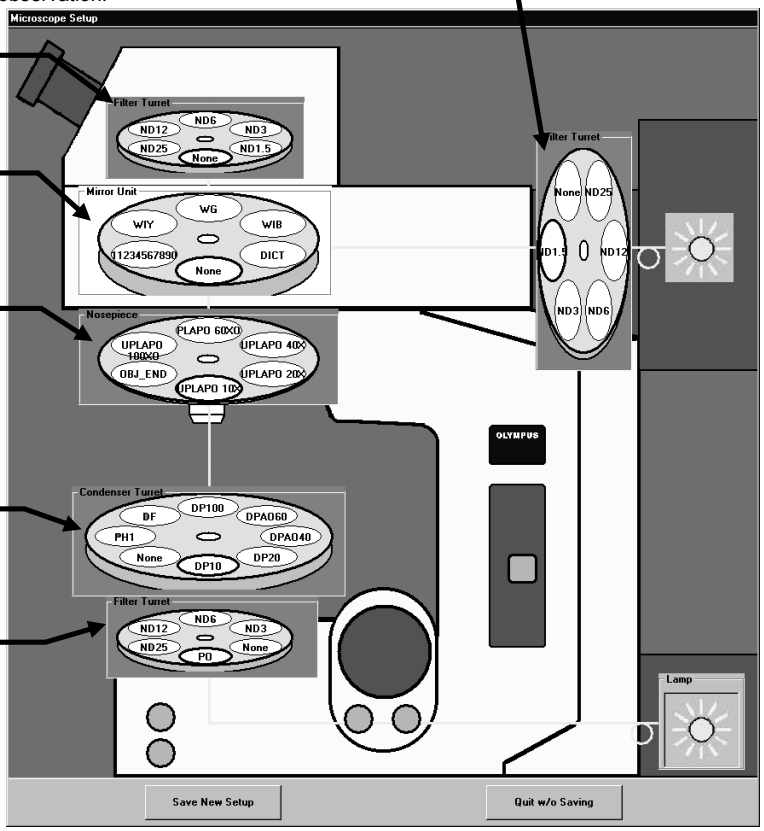
Sets up the name, color of the objective and sets the magnification, N.A, refractive index (n), number of the confocal pinhole, and the condenser worked with to each objective.

[Condenser Turret] group box

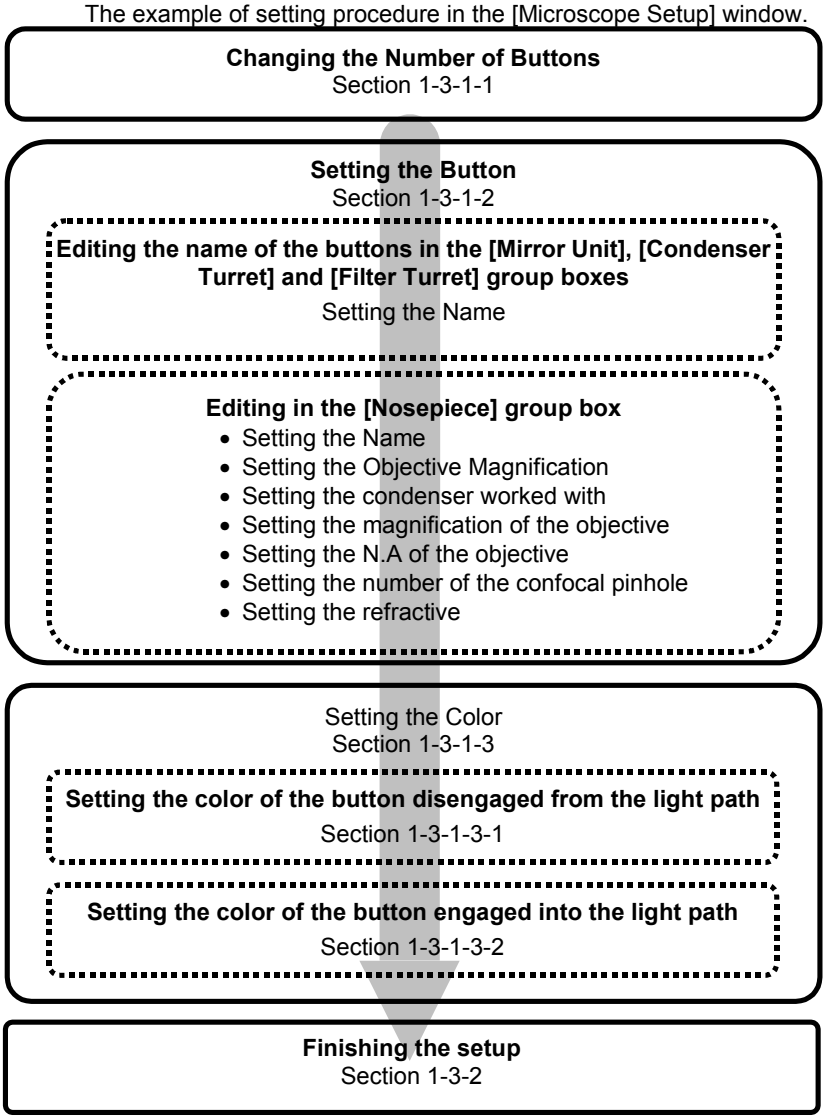
Sets up the name and color of the universal condenser.

[Filter Turret] group box

Sets up the name and color of the filter turret for transmitted observation.

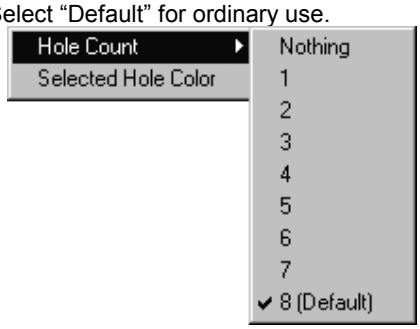


The example of setting procedure in the [Microscope Setup] window.



1-3-2-1 Setting the Number of Buttons

1. Right-click the mouse on the area outside of the buttons in the group box where the number of buttons to be displayed is changed. The pop-up menu as shown below appears. Select “Hole Count” and select the number of the buttons to be displayed in the sub-menu. Select “Default” for ordinary use.

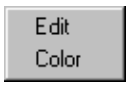


TIP Selecting “Nothing” disables the turret itself.

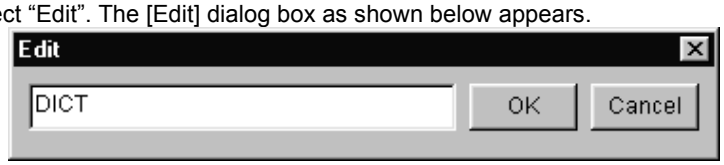
1-3-2-2 Setting the Name

1 Editing the name of the buttons in the [Mirror Unit], [Condenser Turret] and [Filter Turret] group boxes

1. In the [Mirror Unit], [Condenser Turret] or [Filter Turret] group box, right-click the mouse on the button whose name is to be edited. The pop-up menu as shown below appears.



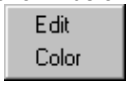
2. Select “Edit”. The [Edit] dialog box as shown below appears.



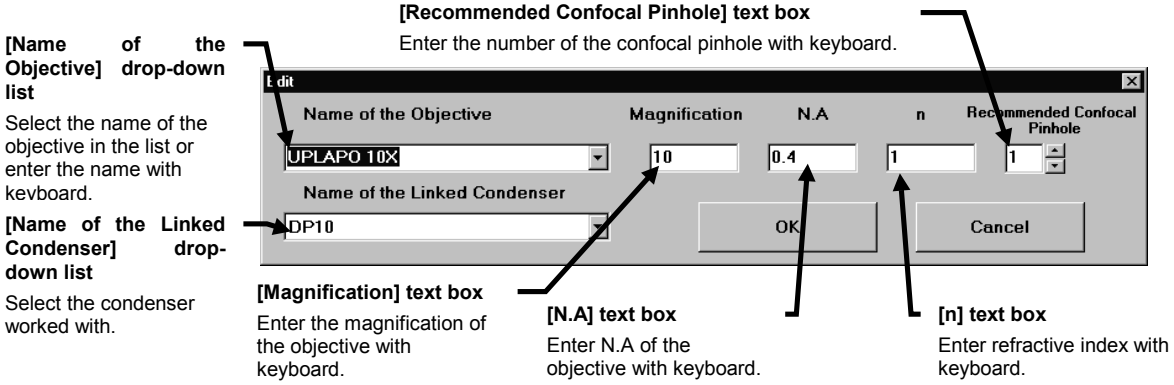
3. Enter a name into the text box and click the <OK> button.

2 Editing in the [Nosepiece] group box

1. In the [Nosepiece] group box, right-click the mouse on the button whose name is to be edited. The pop-up menu as shown below appears.



2. Select “Edit”. The [Edit] dialog box as shown below appears.



3. Click the <OK> button after setting is completed.

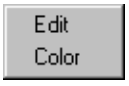
TIP

The initial values are automatically set in the [Magnification], [N.A], [n], and [Recommended Cofocal Pinhole] text boxes after the objective is selected in the [Name of the Objective] drop-down list.

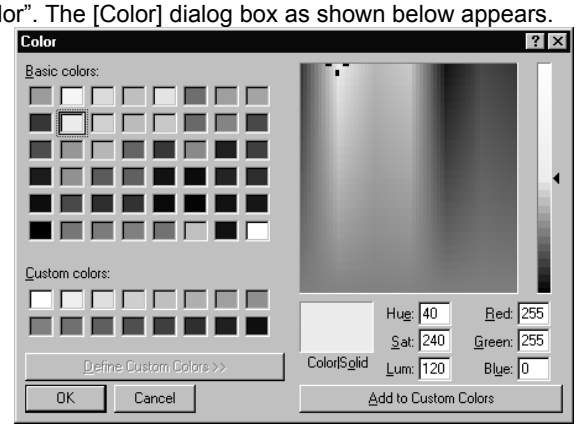
1-3-2-3 Setting the Color

1 Setting the color of the button disengaged from the light path

1. Right-click the mouse on the button whose color is to be changed. The pop-up menu as shown below appears.



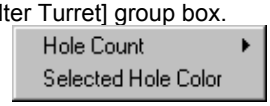
2. Select “Color”. The [Color] dialog box as shown below appears.



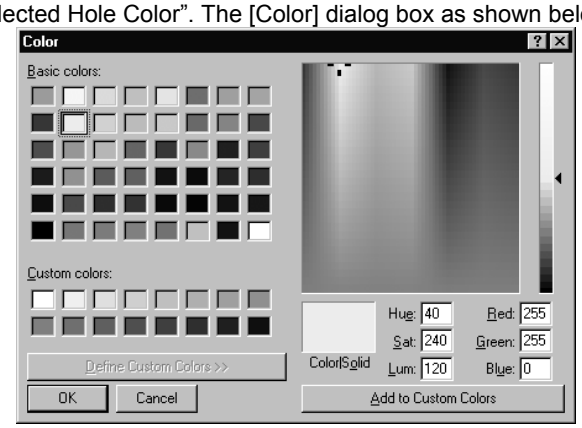
3. Select the color which you want to set in the color palette and click the <OK> button.

2 Setting the color of the button engaged into the light path

1. In [BX Control Panel] window on the software, specify the color of the button of the cube, the objective, and the condenser engaged into the light path. Right-click the mouse on the area outside of the buttons in the [Mirror Unit], [Nosepiece], [Condenser Turret], or [Filter Turret] group box.



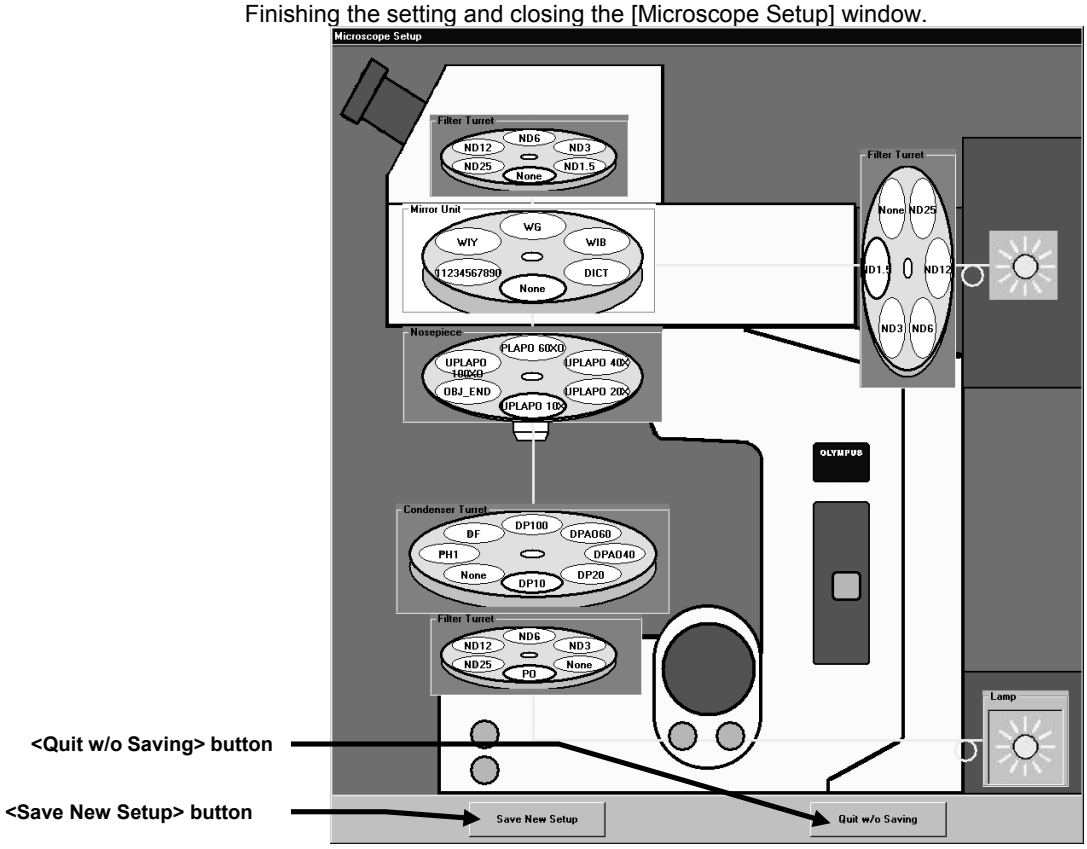
2. Select “Selected Hole Color”. The [Color] dialog box as shown below appears.



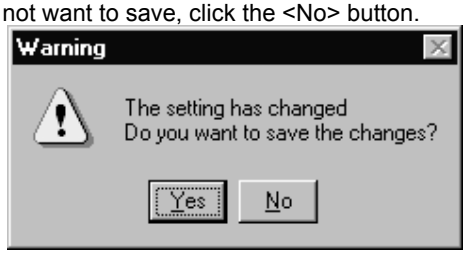
3. Select the color which you want to set in the color palette and click the <OK> button.

1-3-2-4 Finishing the setting

Finishing the setting and closing the [Microscope Setup] window.



1. Click the < Save New Setup> button to save the setup and close the window.
   Or click the <Quit w/o Saving> button to cancel the setup and close the window. Then the dialog box as shown below appears. If you want to save, click the <Yes> button, if you do not want to save, click the <No> button.



NOTE **After saving is selected in the [Microscope Setting] window, the original setting is not returned even if the <Quit w/o Saving> is clicked in the [FLUOVIEW Setup] dialog box.**

1-4 Adding the dyeing method

Newly add a dyeing method and set laser type, excitation wavelength, and emission wavelength.

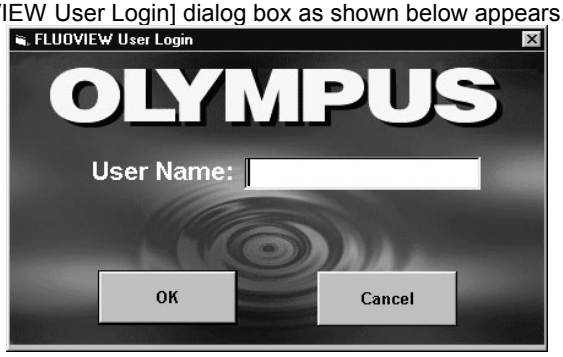
This section describes a simple example of adding CFP as a dyeing method and setting laser type, excitation wavelength, and emission wavelength.

Add the dyeing method using the [Fluorescence] panel in the [Fluoview Setup] dialog box.



[FLUOVIEW Setup] icon

1. Double-click the [FLUOVIEW Setup] icon on the desktop.
   The [FLUOVIEW User Login] dialog box as shown below appears.

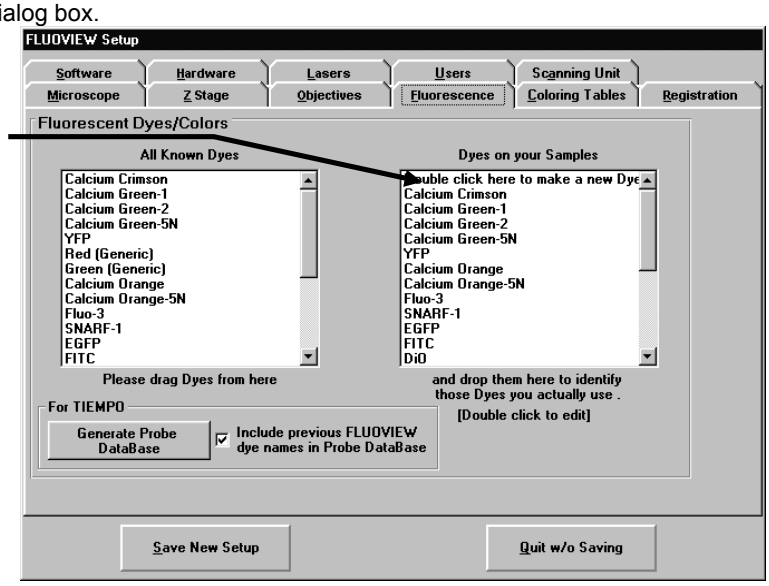


2. Enter user name into the [User Name:] text box and click the <OK> button to log into FLUOVIEW FV500.

TIP When using the system for one user, the [FLUOVIEW User Login] dialog box does not appear.

3. Display the [Fluorescence] panel at the front position in the [FLUOVIEW Setup] dialog box.

Double-click here to add the dyeing method.

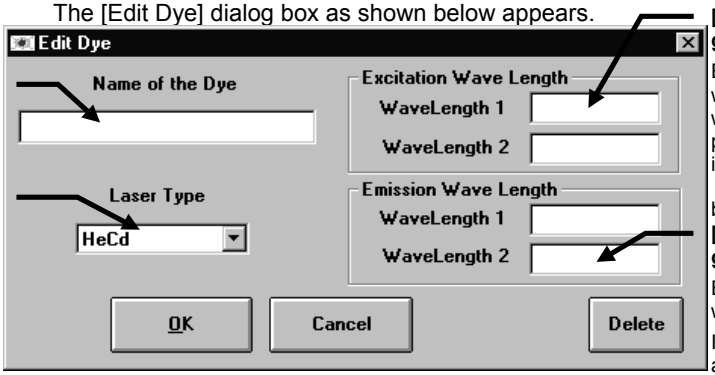


4. Double-click the “Double click here to make a new Dye” in the [Dyes on your Samples] list.

   The [Edit Dye] dialog box as shown below appears.

[[Name of the Dye] text box
Enter the name of the dyeing method.

[Laser Type] drop-down list
Select the laser to use.



[Excitation Wave Length] group box
Enter the excitation wavelength. In case of the 2-wavelength and 1-photometry, enter the value into the [[WaveLength2] text box

[Emission Wave Length] group box
Enter the emission wavelength.
In case of the 1-wavelength and 2-photometry, enter the value into the [[WaveLength2] text box too.

5. Enter the name of the dyeing method into the [Name of the Dye] text box (e,g, CFP).

6. Select the laser to use using the [Laser Type] drop-down list (e.g. HeCd).

TIP

As the laser to use, helium-cadmium laser should be set in advance. When “HeCd” is not displayed, check the [HeCd] check box in the [Laser Equipment] panel.

7. Enter the value of the excitation wavelength into the [WaveLength 1] text box in the [Excitation Wave Length] group box (e.g. 442).

8. Enter the value of the emission wavelength into the [WaveLength 1] text box in the [Emission Wave Length] group box (e.g. 480).

TIP

photometry, enter the value of the wavelength into the [WaveLength 2] text box too.

9. Click the <OK> button to close the [Edit Dye] dialog box.

10. Select the <Save New Setup> button to close the [FLUOVIEW Setup] dialog box.

2 Maintenance of Major System Units

This section describes the maintenance of the Microscope.

2-1 Laser Scanning Microscope

According to IEC60825 “Safety of Laser Product” and IEC60825(EN60825), this product is a CLASS 3B laser product. Therefore, all of activities such as attachment or removal of the microscope modules listed below are defined as the service activities.

I In the United States of America, this product is classified as a CLASS Ⅲb laser product according to the CDRH laser safety regulation, and attachment or removal of the microscope modules listed below is not permitted as part of user maintenance activities. (Such attachment and removal should be performed as part of service activities.)

For detailed imformation, refer to our service representatives.

1) Transmitted lamp housing
2) Transmitted light fiber cable
3) Cube turret of reflected light fluorescence unit
4) Objective revolving nosepiece
5) Objectives
6) Condenser
7) Differential interference slider
8) Excitation filter, spectral filter and barrier filter(Inside the scan unit)

The definitions of the term “maintenance” by IEC(EN60825) are quoted below for reference.

EN60825
Maintenance:
The performance of those adjustments or procedures specified in user information provided by the manufacturer with the laser product, which are to be performed by the user for the purpose of assuring the intended performance of the product. It does not include operation or service.

The definitions of the term “maintenance” by CDRH 21CFR are quoted below for reference.

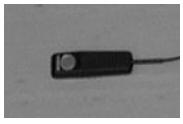
CDRH

“Maintenance” means performance of those adjustments or procedures specified in user information provided by the manufacturer with the laser product which are to be performed by the user for the purpose of assuring the intended performance of the product. It does not include operation or service as defined in paragraph (b) (27) and (38) of this section.

2-2 UV-Ar Laser

2-2-1 Auto Alignment Operation (Remote Switch)

The UV Ar laser is provided with an auto alignment facility, which automatically optimizes the laser oscillation by adjusting the unstability in output due to mirror alignment error resulting from long period of use. It is recommended to perform auto alignment about once a month.



Remote Switch

After warming up the laser head for more than 30 minutes, press the remote switch connected to the rear of the laser head once. The auto alignment starts and completes in some tens of seconds. The indicator on the rear of the laser head blinks during auto alignment.

Do not press the remote switch while the indicator is blinking.

Do not use the remote switch too frequently. The recommend interval is once every few months provided that the UV Ar laser is used frequently. When the UV Ar laser has not been used for a long period, press it only once at the time of idle running of the laser head (aging) which should be performed every month.

2-2-2 Checking, Refilling and Replacing Water in Chiller

1 Checking

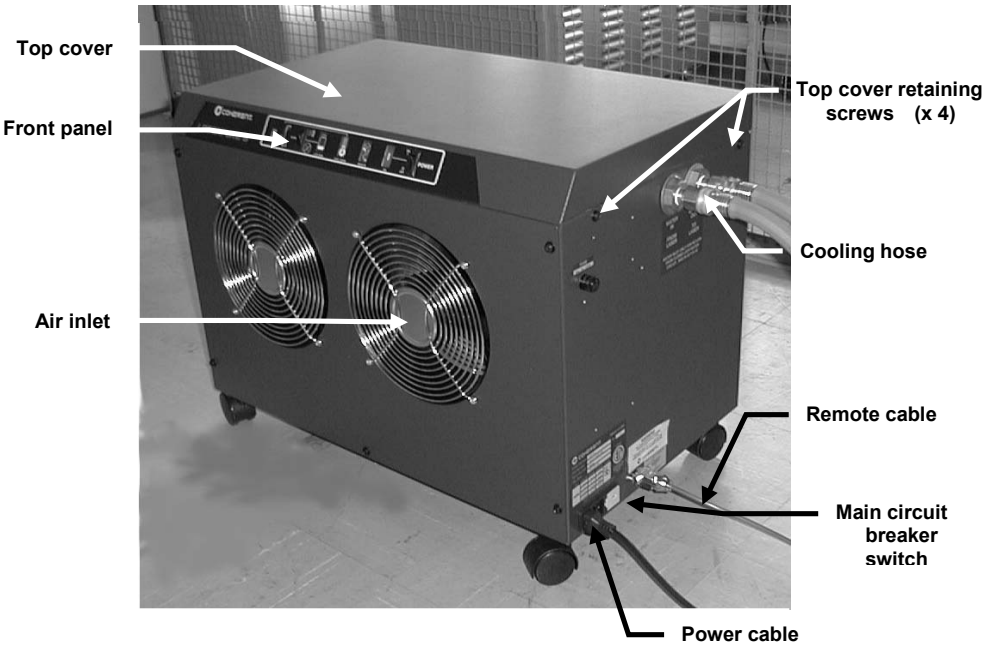
Chiller is a water-cooled system.

After long hours of use, check the water level and, if water is insufficient, **refill distilled water to the specified level.**

If the water in the tank is polluted, change the distilled water.

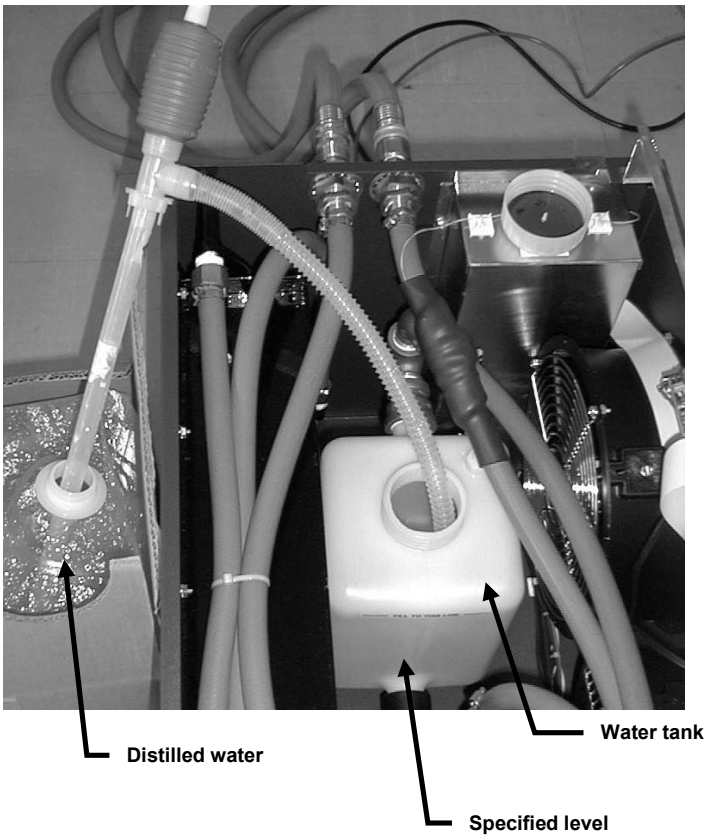
The check period is about once every six months. Note that the water tends to be polluted when the weather is warm or when the heat exchanger has been left unused for a long period.

1. Switch off the main circuit breaker of the heat exchanger and unplug its power cable from the power outlet.



2. Remove the 4 top cover retaining screws and remove the top cover.

3. Remove the water tank cover and check the level and pollution of water in it.



2 Refill and Replacement

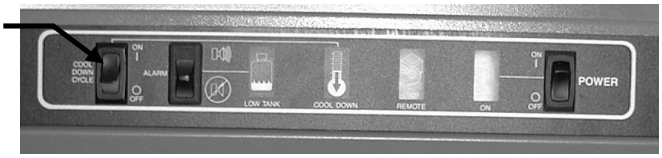
If the water level in the water tank is below the specified level, refill distilled water until the specified level.

If the water is polluted by algae or moth, replace the water to ensure cooling.

Replacement procedure

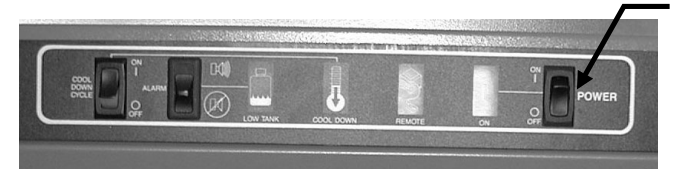
1. Set [COOL DOWN CYCLE] to “O-OFF”.

COOL DOWN CYCLE
Set to “O-OFF”.



2. Pump water out.
3. To eliminate water remaining in the cooling hose, switch [POWER] of the heat exchanger “I-ON”, run it idle and pump out water which comes inside the tank.

POWER
Set to “I-ON”.



4. Refill new distilled water until the specified level. Run the heat exchanger manually and check the water level again. If it is below the specified level, refill additional distilled water.
5. Close the water tank with the lid, place the top cover and tighten the 4 retaining screws tightly using a screwdriver.
6. Plug the power cord into the power outlet and set the main circuit breaker to ON.
7. Set up for normal operation.
   For details, see section 1-2-1, “Turning Power ON” in Volume [OPERATION INSTRUCTIONS].

Consumable supply: Distilled water, about 10 liters (commercially available in 20-liter bottles).

TROUBLESHOOTING

On This Volume

This volume describes the treatment against possible troubles.

In case of trouble, please read volume before calling for service. If the normal operation cannot still be restored, please contact your local Olympus representative.

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1 TROUBLESHOOTING GUIDE

Under certain conditions, performance of this system may be adversely affected by factors other than defects. If a problem occurs, please review the following list and take remedial action as needed.
If you cannot solve the problem after checking the entire list, please contact your local Olympus representative for assistance.
Before contacting Olympus, please fill the “Inquiry Table” at the end of this volume and inform Olympus of its contents.

Phenomenon	Cause	Treatment	Manual Ref. Pages
1. Fluorescence image cannot be observed	Laser is not oscillating.	Turn the laser unit ON. Ensure that the emission key is set to the ON position.	1-2-1, OPERATION INSTRUCTIONS
	An inverted microscope is used and the light path selector dial is not set to the LSM light path. (When an erecting microscope is used, starting scanning sets the LSM light path automatically.	Set the light path selector dial to the LSM light path.	1-1-1 & 1-2-4, OPERATION INSTRUCTIONS
	An excitation tube or analyzer for visual observation is engaged in the microscope. (When the AX70A is used, starting scanning disengages the excitation cube from the light path automatically.)	Disengage the excitation tube and analyzer from the light path.	1-1-1 & 1-2-4, OPERATION INSTRUCTIONS
	The objective is not engaged in the light path.	Stop the objective at a click position. When the AX70A is used, engage the objective correctly in the light path.	Instruction manual of microscope
	The pinhole diameter is small.	Increase the pinhole size.	1-3-2-5, OPERATION INSTRUCTIONS
	The barrier filter is not optimum.	Set the barrier filter.	1-2-6 & 1-3-2-4, OPERATION INSTRUCTIONS
	The light path is blocked by the light path detection switch.	Stop the light path detection switch at a click position.	1-1-1, OPERATION INSTRUCTIONS
	The excitation light is weak.	Increase the transmittance of the ND filter.	1-2-7 & 1-3-2-4, OPERATION INSTRUCTIONS

Phenomenon	Cause	Treatment	Manual Ref. Pages
1. Fluorescence image cannot be observed	The fluorescent dye and excitation wavelength does not match.	Select a laser suitable for the fluorescent dye method. Change the ND filters by reading 1-2-7, “Selecting the ND Filters” in OPERATION INSTRUCTIONS.	1-2-7, OPERATION INSTRUCTIONS
	The fluorescent dye does not match the excitation dichroic mirror, dichroic mirror and/or barrier filter.	Select the barrier filter matching the fluorescent dye method. See 1-3-2-3, “Configuring the Filters” in OPERATION INSTRUCTIONS and follow instructions of the [Optical System Configuration] window.	1-2-6 & 1-3-2-4, OPERATION INSTRUCTIONS
	Focusing is not correct.	Adjust focusing.	1-2-3, OPERATION INSTRUCTIONS
	The photomultiplier tube voltage of the detector is low.	Increase the photomultiplier tube voltage using the [PMT] scale in the [Acquire] panel.	2-2-1-4-9, OPERATION INSTRUCTIONS
	The OFFSET value is too high.	Decrease OFFSET to an optimum value.	2-2-1-4-9, OPERATION INSTRUCTIONS
	The photomultiplier tube of the channel set with the detection mode setting slider is not set.	Check the [Ch1], [Ch2] , [Ch3] , [Ch4] or [Ch5] check box in the [Acquire] panel.	1-2-8-3 & 2-2-1-4-3, OPERATION INSTRUCTIONS
2. Transmitted image cannot be observed.	The transmittance detection channel is not selected.	In the [Acquire] panel, check the check box of the [Ch] with which “Transmitted” is displayed.	2-2-3-3, OPERATION INSTRUCTIONS
	The transmitted light filter is engaged in the light path of the microscope.	Disengage the filter from the light path.	1-3-2-4, OPERATION INSTRUCTIONS
3. Image is disturbed.	The installation location is subject to large vibrations.	Consult Olympus.	-
	External light from a fluorescent lamp, etc. is detected.	Darken the room before acquiring image.	-
		Ensure that the top cover of the scan unit is closed tightly.	-
4. Image is striped.	The laser reflection light is penetrating.	The barrier filter selection is erroneous.	1-2-6 & 1-3-2-4, OPERATION INSTRUCTIONS
5. Image looks poor.	The analyzer is engaged in the light path.	Disengage the analyzer from the light path.	1-2-4, OPERATION INSTRUCTIONS
	The scan speed is high.	Decrease the scan speed to an optimum speed or integrate the image.	1-2-8-9 & 2-2-1-6, OPERATION INSTRUCTIONS

Phenomenon	Cause	Treatment	Manual Ref. Pages
5. Image looks poor.	The fluorescent dye does not match the barrier filter.	Select the barrier filter matching the fluorescent dye. Change the ND filters by reading 1-2-7, “Selecting the ND Filter” in OPERATION INSTRUCTIONS.	1-2-7, OPERATION INSTRUCTIONS
	The excitation light is weak.	Increase the transmittance of the ND filter.	1-2-7, OPERATION INSTRUCTIONS
	The installation location is subject to large vibrations.	Consult Olympus.	-
6. Image is irregularly blurred or the brightness is uneven.	The specimen is tilted.	Set the specimen horizontally.	Instruction manual of microscope
7. Image is flared.	Non-fluorescent glass is not used.	Use non-fluorescent glass.	-
	The cover glass thickness is not optimum.	Use a cover glass with a thickness of 0.17 mm.	-
	The specimen is over-stained.	Stain it properly again or increase the OFFSET value.	2-2-1-4-9, OPERATION INSTRUCTIONS
8. Circular flare is observed at the center of image.	The excitation wavelength does not match the barrier filter type or the barrier filter is not used.	Select suitable barrier filter for the excitation length.	1-2-6 & 1-3-2-4, OPERATION INSTRUCTIONS
9. Image is blurred or out of focus.	The focusing is not correct.	Adjust focusing with visual observation.	Instruction manual of microscope
10. Image is dark and noisy.	The fluorescent dye is pale.	Apply optimum fluorescent dye.	-
	The pinhole diameter is too small.	Adjust to an optimum size.	1-3-2-5, OPERATION INSTRUCTIONS
	The HV of photomultiplier tube exceeds 800.	Set HV at no more than 800. If the image is still dark, adjust GAIN.	2-2-1-4-9, OPERATION INSTRUCTIONS
	The scan speed is too high.	Decrease the scan speed or integrate the image.	1-2-8-9 & 2-2-1-6, OPERATION INSTRUCTIONS
	The excitation light is weak.	Increase the transmittance of the ND filter.	1-2-7, 1-3-2-4 & 2-2-1-4-10, OPERATION INSTRUCTIONS
11. The position reproduction of the Z-motor is poor.	The Z-position fluctuates because the coarse movement knob of microscope is too light.	Make the microscope’s coarse movement knob heavier by turning the microscope’s rotation tension adjustment ring.	Instruction manual of microscope

Phenomenon	Cause	Treatment	Manual Ref. Pages
12. The [Acquire] panel cannot be displayed.	The power unit is not recognized.	Check the connection between the computer and power unit. Turn the power unit ON.	1-2-1, OPERATION INSTRUCTIONS
	If the combination with the AX70A is used, the microscope is not recognized.	Check the connections of the PC and microscope. Turn the microscope on.	1-2-1, OPERATION INSTRUCTIONS
13. The scale of the [Z Stage] sub-panel in the [Acquire] panel cannot be moved.	The Z-stage (Z revolving nosepiece) motor is not excited.	Check the [Locked] check box in the [Z Stage] sub-panel.	2-2-1-4-7, OPERATION INSTRUCTIONS
14. The fine movement knob of microscope cannot be turned or is not smooth.	The Z-stage (Z revolving nosepiece) motor is excited.	Clear the [Locked] check box in the [Z Stage] sub-panel.	2-2-1-4-7, OPERATION INSTRUCTIONS
15. The image cannot be saved in the disk.	The disk drive is not ON.	Ensure that the MO disk drive is ON	Instruction manual of MO disk
	The MO disk is not recognized.	Check the cable connection.	Instruction manual of MO disk
	The disk is not loaded in the drive.	Insert the disk properly.	Instruction manual of MO disk
	The disk is write-protected.	Release the write protection.	Instruction manual of MO disk
	The disk is not formatted. (either floppy disk or MO disk).	Format the disk before use.	Appendix F, OPERATION INSTRUCTIONS
	The available space in disk is insufficient.	Increase the available space in disk by deleting unnecessary files or use another disk (be sure to format a new disk).	- Appendix F, OPERATION INSTRUCTIONS
16. The image cannot be output at the printer.	The printer is not recognized.	Ensure that the printer is ON.	Instruction manual of printer
	The printer is not recognized.	Check the cable connection.	Instruction manual of printer
	The printer is out of paper.	Supply paper.	Instruction manual of printer
17. The FLUOVIEW software cannot be started.	The FLUOVIEW software has already been started.	Press Alt + Tab on the keyboard to switch to FLUOVIEW.	1-2-2, OPERATION INSTRUCTIONS
	Another application is running.	Exit from the running software before starting the FLUOVIEW software.	

Phenomenon	Cause	Treatment	Manual Ref. Pages
18. The mouse pointer in the screen cannot be moved by moving the mouse.	The software is malfunctioning.	Press Ctrl + Alt + Delete on the keyboard and exit from FLUOVIEW by following the displayed messages. Then exit from windows and reboot the computer.	1-2-1 & 1-2-11, OPERATION INSTRUCTIONS
19. The power supply of Power Unit(FV5-PSU) is not turned on.	The fuse has run out.	Contact your local Olympus representative for assistance then replace the huse.	-
20. The current limit indicator on the UV Ar laser power supply is blinking.	The laser is in standby condition.	Start the FLUOVIEW software, then click the <On> button for [UV Ar Laser] in the [Laser Unit] group box in the [Optical System Configuration] window to restart laser oscillation.	1-3-2-4, OPERATION INSTRUCTIONS
21. The error indicator on the UV Ar laser power supply will not turn off.	-	Consult your local Olympus representative.	
22. Bios some check error message appears at the moment of starting up the computer.	The battery maintains Bios set value has run out.	Contact your local Olympus representative for assistance to replace the battery.	-
	Bios has broken.	Consult Olympus.	-

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